

Practitioner's Docket No. P-1067

526 Rec'd PCT/PTO 10 JUL 2001

CHAPTER II

09/889015

## Preliminary Classification:

Proposed Class:

Subclass:

NOTE: "All applicants are requested to include a preliminary classification on newly filed patent applications. The preliminary classification, preferably class and subclass designations, should be identified in the upper right-hand corner of the letter of transmittal accompanying the application papers, for example 'Proposed Class 2, subclass 129.' " M.P.E.P., § 601, 7th ed.

**TRANSMITTAL LETTER  
TO THE UNITED STATES ELECTED OFFICE (EO/US)  
(ENTRY INTO U.S. NATIONAL PHASE UNDER CHAPTER II)**

PCT/EP 99/10088	17 Dec. 1999	12 Jan. 1999
INTERNATIONAL APPLICATION NO.	INTERNATIONAL FILING DATE	PRIORITY DATE CLAIMED
MYCOTOXIN ADSORBENT		
TITLE OF INVENTION		
Dr. Norbert Schall; Hubert Simmler-Hübenthal; Herrmann Gerardo Feldhaus		
APPLICANT(S)		

**Box PCT**  
**Assistant Commissioner for Patents**  
**Washington D.C. 20231**  
**ATTENTION: EO/US**

**CERTIFICATION UNDER 37 C.F.R. §§ 1.8(a) and 1.10\***

(When using Express Mail, the Express Mail label number is **mandatory**;  
Express Mail certification is optional.)

I hereby certify that, on the date shown below, this correspondence is being:

**MAILING**

☒ deposited with the United States Postal Service in an envelope addressed to the Assistant Commissioner for Patents, Washington, D.C. 20231

37 C.F.R. § 1.8(a)

☐ with sufficient postage as first class mail.

37 C.F.R. § 1.10 \*

☒ as "Express Mail Post Office to Addressee"

Mailing Label No. **EK985526179US** (mandatory)

**TRANSMISSION**

☐ facsimile transmitted to the Patent and Trademark Office, (703) \_\_\_\_\_

Signature

Dorothy Goodlett

(type or print name of person certifying)

Date: July 10, 2001

\* Only the date of filing (§ 1.6) will be the date used in a patent term adjustment calculation, although the date on any certificate of mailing or transmission under § 1.8 continues to be taken into account in determining timeliness. See § 1.703(f). Consider "Express Mail Post Office to Addressee" (§ 1.10) or facsimile transmission (§ 1.6(d)) for the reply to be accorded the earliest possible filing date for patent term adjustment calculations.

(Transmittal Letter to the United States Elected Office (EO/US) [13-18]—page 1 of 9)

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**NOTE:** To avoid abandonment of the application, the applicant shall furnish to the USPTO, not later than 20 months from the priority date: (1) a copy of the international application, unless it has been previously communicated by the International Bureau or unless it was originally filed in the USPTO; and (2) the basic national fee (see 37 C.F.R. § 1.492(a)). The 30-month time limit may not be extended. 37 C.F.R. § 1.495.

**WARNING:** Where the items are those which can be submitted to complete the entry of the international application into the national phase are subsequent to 30 months from the priority date the application is still considered to be in the international state and if mailing procedures are utilized to obtain a date the express mail procedure of 37 C.F.R. § 1.10 must be used (since international application papers are not covered by an ordinary certificate of mailing—See 37 C.F.R. § 1.8.

**NOTE:** Documents and fees must be clearly identified as a submission to enter the national state under 35 U.S.C. § 371 otherwise the submission will be considered as being made under 35 U.S.C. § 111. 37 C.F.R. § 1.494(f).

- I. Applicant herewith submits to the United States Elected Office (EO/US) the following items under 35 U.S.C. § 371:
- a. ☒ This express request to immediately begin national examination procedures (35 U.S.C. § 371(f)).
  - b. ☒ The U.S. National Fee (35 U.S.C. § 371(c)(1)) and other fees (37 C.F.R. § 1.492) as indicated below:

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\*See attached Preliminary Amendment Reducing the Number of Claims.

- ☒ Attached is a ☒ check ☐ money order in the amount of \$ 1,226.00
- ☒ Authorization is hereby made to charge the amount of \$
- ☒ to Deposit Account No. 03-3420
- ☐ to Credit card as shown on the attached credit card information authorization form PTO-2038.

**WARNING:** Credit card information should **not** be included on this form as it may become public.

- ☒ Charge any additional fees required by this paper or credit any overpayment in the manner authorized above.

A duplicate of this paper is attached.

**\*\*WARNING:** "To avoid abandonment of the application the applicant shall furnish to the United States Patent and Trademark Office not later than the expiration of 30 months from the priority date: \* \* \* (2) the basic national fee (see § 1.492(a)). The 30-month time limit may not be extended." 37 C.F.R. § 1.495(b).

**WARNING:** If the translation of the international application and/or the oath or declaration have not been submitted by the applicant within thirty (30) months from the priority date, such requirements may be met within a time period set by the Office. 37 C.F.R. § 1.495(b)(2). The payment of the surcharge set forth in § 1.492(e) is required as a condition for accepting the oath or declaration later than thirty (30) months after the priority date. The payment of the processing fee set forth in § 1.492(f) is required for acceptance of an English translation later than thirty (30) months after the priority date. Failure to comply with these requirements will result in abandonment of the application. The provisions of § 1.136 apply to the period which is set. Notice of Jan. 3, 1993, 1147 O.G. 29 to 40.

☐ **Assertion of Small Entity Status**

☐ **Applicant hereby asserts status as a small entity under 37 C.F.R. § 1.27.**

**NOTE:** 37 C.F.R. § 1.27(c) deals with the assertion of small entity status, whether by a written specific declaration thereof or by payment as a small entity of the basic filing fee or the fee for the entry into the national phase as states:

"(c) Assertion of small entity status. Any party (person, small business concern or nonprofit organization) should make a determination, pursuant to paragraph (f) of this section, of entitlement to be accorded small entity status based on the definitions set forth in paragraph (a) of this section, and must, in order to establish small entity status for the purpose of paying small entity fees, actually make an assertion of entitlement to small entity status, in the manner set forth in paragraphs (c)(1) or (c)(3) of this section, in the application or patent in which such small entity fees are to be paid.

(1) Assertion by writing. Small entity status may be established by a written assertion of entitlement to small entity status. A written assertion must:

(i) Be clearly identifiable;

(ii) Be signed (see paragraph (c)(2) of this section); and

(iii) Convey the concept of entitlement to small entity status, such as by stating that applicant is a small entity, or that small entity status is entitled to be asserted for the application or patent. While no specific words or wording are required to assert small entity status, the intent to assert small entity status must be clearly indicated in order to comply with the assertion requirement.

(2) Parties who can sign and file the written assertion. The written assertion can be signed by:

(i) One of the parties identified in §§ 1.33(b) (e.g., an attorney or agent registered with the Office), §§ 3.73(b) of this chapter notwithstanding, who can also file the written assertion;

(ii) At least one of the individuals identified as an inventor (even though a § 1.63 executed oath or declaration has not been submitted), notwithstanding §§ 1.33(b)(4), who can also file the written assertion pursuant to the exception under §§ 1.33(b) of this part; or

(iii) An assignee of an undivided part interest, notwithstanding §§ 1.33(b)(3) and 3.73(b) of this chapter, but the partial assignee cannot file the assertion without resort to a party identified under §§ 1.33(b) of this part.

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## 2. Fees

CLAIMS FEE	(1) FOR	(2) NUMBER FILED	(3) NUMBER EXTRA	(4) RATE	(5) CALCULATIONS
<input type="checkbox"/> *	TOTAL CLAIMS				
	27	-20=	7	× \$18.00=	\$ 126.00
	INDEPENDENT CLAIMS				
	6	-3=	3	× \$80.00=	240.00
	MULTIPLE DEPENDENT CLAIM(S) (if applicable) + \$270.00				
BASIC FEE**	<input type="checkbox"/> U.S. PTO WAS INTERNATIONAL PRELIMINARY EXAMINATION AUTHORITY Where an International preliminary examination fee as set forth in § 1.482 has been paid on the international application to the U.S. PTO: <ul style="list-style-type: none"> <li><input type="checkbox"/> and the international preliminary examination report states that the criteria of novelty, inventive step (non-obviousness) and industrial activity, as defined in PCT Article 33(1) to (4) have been satisfied for all the claims presented in the application entering the national stage (37 C.F.R. § 1.492(a)(4)) ..... \$100.00</li> <li><input type="checkbox"/> and the above requirements are not met (37 C.F.R. § 1.492(a)(1)) ..... \$690.00</li> </ul> <input checked="" type="checkbox"/> U.S. PTO WAS NOT INTERNATIONAL PRELIMINARY EXAMINATION AUTHORITY Where no international preliminary examination fee as set forth in § 1.482 has been paid to the U.S. PTO, and payment of an international search fee as set forth in § 1.445(a)(2) to the U.S. PTO: <ul style="list-style-type: none"> <li><input type="checkbox"/> has been paid (37 C.F.R. § 1.492(a)(2)) ..... \$710.00</li> <li><input type="checkbox"/> has not been paid (37 C.F.R. § 1.492(a)(3)) ..... \$1000.00</li> <li><input checked="" type="checkbox"/> where a search report on the international application has been prepared by the European Patent Office or the Japanese Patent Office (37 C.F.R. § 1.492(a)(5)) ..... \$860.00</li> </ul>				
	Total of above Calculations				= \$1,226.00
SMALL ENTITY	Reduction by 1/2 for filing by small entity, if applicable. Assertion must be made. (note 37 C.F.R. § 1.27)				-
	Subtotal				\$1,226.00
	Total National Fee				\$ 1,226.00
	Fee for recording the enclosed assignment document \$40.00 (37 C.F.R. § 1.21(h)). (See Item 13 below). See attached "ASSIGNMENT COVER SHEET".				
TOTAL	Total Fees enclosed				\$ 1,226.00

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(3) Assertion by payment of the small entity basic filing or basic national fee. The payment, by any party, of the exact amount of one of the small entity basic filing fees set forth in §§ 1.16(a), (f), (g), (h), or (k), or one of the small entity basic national fees set forth in §§ 1.492(a)(1), (a)(2), (a)(3), (a)(4), or (a)(5), will be treated as a written assertion of entitlement to small entity status even if the type of basic filing or basic national fee is inadvertently selected in error.

(i) If the Office accords small entity status based on payment of a small entity basic filing or basic national fee under paragraph (c)(3) of this section that is not applicable to that application, any balance of the small entity fee that is applicable to that application will be due along with the appropriate surcharge set forth in §§ 1.16(e), or §§ 1.16(f).

(ii) The payment of any small entity fee other than those set forth in paragraph (c)(3) of this section (whether in the exact fee amount or not) will not be treated as a written assertion of entitlement to small entity status and will not be sufficient to establish small entity status in an application or a patent."

3. ☒ A copy of the International application as filed (35 U.S.C. § 371(c)(2)):

NOTE: Section 1.495 (b) was amended to require that the basic national fee and a copy of the international application must be filed with the Office by 30 months from the priority date to avoid abandonment. "The International Bureau normally provides the copy of the international application to the Office in accordance with PCT Article 20. At the same time, the International Bureau notifies applicant of the communication to the Office. In accordance with PCT Rule 47.1, that notice shall be accepted by all designated offices as conclusive evidence that the communication has duly taken place. Thus, if the applicant desires to enter the national stage, the applicant normally need only check to be sure the notice from the International Bureau has been received and then pay the basic national fee by 30 months from the priority date." Notice of Jan. 7, 1993, 1147 O.G. 29 to 40, at 35-36. See item 14c below.

- a. ☐ is transmitted herewith.
- b. ☐ is not required, as the application was filed with the United States Receiving Office.
- c. ☒ has been transmitted
  - i. ☒ by the International Bureau.

Date of mailing of the application (from form PCT/1B/308):

- ii. ☐ by applicant on \_\_\_\_\_. (Date)

4. ☒ A translation of the International application into the English language (35 U.S.C. § 371(c)(2)):

- a. ☒ is transmitted herewith.
- b. ☐ is not required as the application was filed in English.
- c. ☐ was previously transmitted by applicant on \_\_\_\_\_. (Date)
- d. ☐ will follow.

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5. ☒ Amendments to the claims of the International application under PCT Article 19 (35 U.S.C. § 371(c)(3)):

**NOTE:** *The Notice of January 7, 1993 points out that 37 C.F.R. § 1.495(a) was amended to clarify the existing and continuing practice that PCT Article 19 amendments must be submitted by 30 months from the priority date and this deadline may not be extended. The Notice further advises that: "The failure to do so will not result in loss of the subject matter of the PCT Article 19 amendments. Applicant may submit that subject matter in a preliminary amendment filed under section 1.121. In many cases, filing an amendment under section 1.121 is preferable since grammatical or idiomatic errors may be corrected." 1147 O.G. 29-40, at 36.*

- a. ☒ are transmitted herewith, **in a preliminary amendment.**  
b. ☐ have been transmitted  
i. ☐ by the International Bureau.

Date of mailing of the amendment (from form PCT/1B/308):

- ii. ☐ by applicant on \_\_\_\_\_. (Date)
- c. ☐ have not been transmitted as
- i. ☐ applicant chose not to make amendments under PCT Article 19.  
Date of mailing of Search Report (from form PCT/ISA/210.):  
\_\_\_\_\_
- ii. ☐ the time limit for the submission of amendments has not yet expired. The amendments or a statement that amendments have not been made will be transmitted before the expiration of the time limit under PCT Rule 46.1.

6. ☒ A translation of the amendments to the claims under PCT Article 19 (38 U.S.C. § 371(c)(3)):
- a. ☐ is transmitted herewith.
  - b. ☒ is not required as the amendments were made in the English language.
  - c. ☐ has not been transmitted for reasons indicated at point 5(c) above.
7. ☒ A copy of the international examination report (PCT/IPEA/409)
- ☒ is transmitted herewith.
  - ☐ is not required as the application was filed with the United States Receiving Office.
8. ☐ Annex(es) to the international preliminary examination report
- a. ☐ is/are transmitted herewith.
  - b. ☐ is/are not required as the application was filed with the United States Receiving Office.
9. ☐ A translation of the annexes to the international preliminary examination report
- a. ☐ is transmitted herewith.
  - b. ☐ is not required as the annexes are in the English language.

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10. ☒ An oath or declaration of the inventor (35 U.S.C. § 371(c)(4)) complying with 35 U.S.C. § 115
- a. ☐ was previously submitted by applicant on \_\_\_\_\_. (Date)
  - b. ☐ is submitted herewith, and such oath or declaration
    - i. ☐ is attached to the application.
    - ii. ☐ identifies the application and any amendments under PCT Article 19 that were transmitted as stated in points 3(b) or 3(c) and 5(b); and states that they were reviewed by the inventor as required by 37 C.F.R. § 1.70.
  - c. ☒ will follow.

II. Other document(s) or information included:

11. ☒ An International Search Report (PCT/ISA/210) or Declaration under PCT Article 17(2)(a):
- a. ☒ is transmitted herewith.
  - b. ☐ has been transmitted by the International Bureau.  
Date of mailing (from form PCT/IB/308): \_\_\_\_\_
  - c. ☐ is not required, as the application was searched by the United States International Searching Authority.
  - d. ☐ will be transmitted promptly upon request.
  - e. ☐ has been submitted by applicant on \_\_\_\_\_. (Date)
12. ☐ An Information Disclosure Statement under 37 C.F.R. §§ 1.97 and 1.98:
- a. ☐ is transmitted herewith.

Also transmitted herewith is/are:

- ☐ Form PTO-1449 (PTO/SB/08A and 08B).
  - ☐ Copies of citations listed.
  - b. ☐ will be transmitted within THREE MONTHS of the date of submission of requirements under 35 U.S.C. § 371(c).
  - c. ☐ was previously submitted by applicant on \_\_\_\_\_. (Date)
13. ☐ An assignment document is transmitted herewith for recording.
- A separate ☐ "COVER SHEET FOR ASSIGNMENT (DOCUMENT) ACCOMPANYING NEW PATENT APPLICATION" or ☐ FORM PTO 1595 is also attached.

14. ☒ Additional documents:

- a. ☒ Copy of request (PCT/RO/101)
- b. ☐ International Publication No. \_\_\_\_\_
- i. ☐ Specification, claims and drawing
- ii. ☐ Front page only
- c. ☐ Preliminary amendment (37 C.F.R. § 1.121)
- d. ☐ Other

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15. ☒ The above checked items are being transmitted

- a. ☒ before 30 months from any claimed priority date.
- b. ☐ after 30 months.

16. ☐ Certain requirements under 35 U.S.C. § 371 were previously submitted by the applicant on \_\_\_\_\_, namely:

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**AUTHORIZATION TO CHARGE ADDITIONAL FEES**

**WARNING:** Accurately count claims, especially multiple dependant claims, to avoid unexpected high charges if extra claims are authorized.

**NOTE:** "A written request may be submitted in an application that is an authorization to treat any concurrent or future reply, requiring a petition for an extension of time under this paragraph for its timely submission, as incorporating a petition for extension of time for the appropriate length of time. An authorization to charge all required fees, fees under § 1.17, or all required extension of time fees will be treated as a constructive petition for an extension of time in any concurrent or future reply requiring a petition for an extension of time under this paragraph for its timely submission. Submission of the fee set forth in § 1.17(a) will also be treated as a constructive petition for an extension of time in any concurrent reply requiring a petition for an extension of time under this paragraph for its timely submission." 37 C.F.R. § 1.136(a)(3).

**NOTE:** "Amounts of twenty-five dollars or less will not be returned unless specifically requested within a reasonable time, nor will the payer be notified of such amounts; amounts over twenty-five dollars may be returned by check or, if requested, by credit to a deposit account." 37 C.F.R. § 1.26(a).

- ☒ Please charge, in the manner authorized above, the following additional fees that may be required by this paper and during the entire pendency of this application:
- ☒ 37 C.F.R. § 1.492(a)(1), (2), (3), and (4) (filing fees)

**WARNING:** Because failure to pay the national fee within 30 months without extension (37 C.F.R. § 1.495(b)(2)) results in abandonment of the application, it would be best to always check the above box.



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- ☒ 37 C.F.R. § 1.492(b), (c) and (d) (presentation of extra claims)

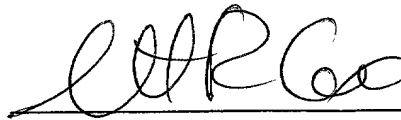
NOTE: Because additional fees for excess or multiple dependent claims not paid on filing or on later presentation must only be paid or these claims cancelled by amendment prior to the expiration of the time period set for response by the PTO in any notice of fee deficiency (37 C.F.R. § 1.492(d)), it might be best not to authorize the PTO to charge additional claim fees, except possible when dealing with amendments after final action.

- ☐ 37 C.F.R. § 1.17 (application processing fees)
- ☐ 37 C.F.R. § 1.17(a)(1)-(5) (extension fees pursuant to § 1.136(a).
- ☐ 37 C.F.R. § 1.18 (issue fee at or before mailing of Notice of Allowance, pursuant to 37 C.F.R. § 1.311(b))

NOTE: Where an authorization to charge the issue fee to a deposit account has been filed before the mailing of a Notice of Allowance, the issue fee will be automatically charged to the deposit account at the time of mailing the notice of allowance. 37 C.F.R. § 1.311(b).

NOTE: 37 C.F.R. § 1.28(b) requires "Notification of any change in loss of entitlement to small entity status must be filed in the application . . . prior to paying, or at the time of paying . . . issue fee." From the wording of 37 C.F.R. § 1.28(b): (a) notification of change of status must be made even if the fee is paid as "other than a small entity" and (b) no notification is required if the change is to another small entity.

- ☐ 37 C.F.R. § 1.492(e) and (f) (surcharge fees for filing the declaration and/or filing an English translation of an International Application later than 30 months after the priority date).



SIGNATURE OF PRACTITIONER

Reg. No.: 31,945

Scott R. Cox

Tel. No.: ( 502 ) 589-4215

(type or print name of practitioner)

400 West Market St., Suite 2200

Customer No.:

P.O. Address

Louisville, KY 40202

09/889015

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## IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of: :  
Dr. Norbert Schall, et al. :  
Serial No. : Art Unit:  
Filing Date: : Examiner:  
Attorney Docket No. P-1067 :  
For: MYCOTOXIN ADSORBENT :

BOX PCT  
Assistant Commissioner for Patents  
Washington, D.C. 20231  
ATTENTION: EO/US

## PRELIMINARY AMENDMENT

In the Specification

The applicants have amended the original translation of the specification from German to English to place the specification in a better condition for review. Attached to this Preliminary Amendment as Exhibit A is an original translation of the application with amendments handwritten on the translation. Also attached as Exhibit B is a rewritten application with these amendments incorporated therein. No new subject matter is introduced by these amendments.

In the Claims

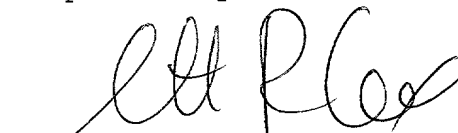
Please cancel all claims of the application, Claims 1-12, as filed and substitute the new claims, Claims 13-39, which are

contained in Exhibit B. The amended claims are written in better form for review by the United States Patent and Trademark Office. No new subject matter is introduced in these new claims.

### CONCLUSION

The applicants believe the application, as amended, with the new claims is in condition for review by the United States Patent and Trademark Office.

Respectfully submitted,



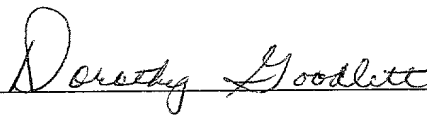
\_\_\_\_\_  
Scott R. Cox  
Reg. No. 31,945  
LYNCH, COX, GILMAN & MAHAN, P.S.C.  
400 West Market Street, Suite 2200  
Louisville, Kentucky 40202  
(502) 589-4215

### CERTIFICATE OF SERVICE

I hereby certify that this correspondence and the documents referred to as attached therein are being deposited with the United States Postal Service as "Express Mail Post Office to Addressee," mailing Label Number **EK985526179US**, addressed to Box PCT, Assistant Commissioner for Patents, Washington, D.C. 20231, Attention: EO/US.

Dated:

July 10, 2001



SRC:dg  
C:\WP\PAT\P1067.PAM  
7-10-01  
410670

~~PATENT APPLICATION~~

### Mycotoxin Adsorbent

Description

The present invention concerns a mycotoxin adsorbent, especially for adsorption of aflatoxins and other mycotoxins (non-aflatoxins) in feeds, cereals, grains and animal feeds.

## ~~Background of the Invention~~

The term mycotoxin encompasses a group of toxic substances that are formed by different naturally occurring fungi. About 300 to 400 mycotoxins are now known. Cereals and grains are generally considered the natural environment for these fungi. Whereas some types of fungi already develop in the spike in the still maturing grain, other types primarily attack grain supplies being stored when a certain minimum moisture and ambient temperature <sup>CONDITIONS</sup> are present.

All so-called mycotoxins have a health-hazardous effect primarily on agricultural animals fed with infected grain, but secondarily on humans as well via the food chain. For example, aflatoxins are responsible for the so-called X-disease of turkeys, which destroyed about 100,000 animals in Great Britain in 1960/61, which had been fed with moldy peanut flour.

Some of the most important mycotoxins are:

Aflatoxins B<sub>1</sub>, B<sub>2</sub>, G<sub>1</sub>, G<sub>2</sub>: these are formed by various *Aspergillus* species. Aflatoxin B<sub>1</sub> is carcinogenic even in microgram amounts and causes stomach and liver damage.

Ochratoxin is formed by *Aspergillus ochraceus* and *Penicillium viridicatum* and causes kidney damage.

Zearalenone is formed by *Fusarium graminearum*, which grows on corn, barley and wheat. It is an estrogen-like substance that causes fertility disorders and is suspected to be carcinogenic.

Fumonisin is formed by fungi of the genus *Fusarium* and has been implicated, among other things, in horse deaths.

T2 toxins and T2-like toxins (tricothecenes) are formed by fungi of the genus *Fusarium*.

Moreover, there are a number of additional mycotoxins, like deoxynivalenol, diacetoxyscirpenol, patuline, citrinine, byssochlamic acid, ochratoxin, sterigmatocystine, moniliformine, ergot alkaloids, ergochrome, cytochalasane, penicillinic acid, zearalenone, rubratoxins, tricothecenes (cf. Römpps, *Chemie-Lexikon*, 8<sup>th</sup> Edition, 1985, page 2888), and others, which occur, ~~however, only in isolated fashion~~ <sup>only in isolated circumstances,</sup> in concentrations that cause health problems in feeds.

Several different toxins that <sup>all</sup> ~~were~~ <sup>can be discovered</sup> recognized as causal agents of health problems in humans and animals ~~could be determined~~ <sup>utilization</sup> in different feeds by the ~~development~~ <sup>have been</sup> of more sensitive analysis methods. A number of studies ~~were~~ <sup>have been</sup> able to demonstrate that several toxins can occur simultaneously in feeds. This simultaneous occurrence can significantly influence the toxicity of the mycotoxins. In addition to acute damage to agricultural animals that receive mycotoxin-contaminated feed, health impairment in humans <sup>has been</sup> ~~is also being~~ discussed in the literature, <sup>which</sup> ~~Such impairment~~ <sup>develops</sup> ~~by~~ <sup>after</sup> long-term intake of foods <sup>even</sup> weakly contaminated with mycotoxins.

In a recent study of suspected feed samples, aflatoxin, deoxynivalenone or fumonisin were found in more than 70% of the investigated samples (cf. "Understanding and Coping with Effects of Mycotoxins in <sup>live</sup> Life Dog Feed and Forage", North Carolina Cooperative Extension Service, North Carolina State University; <http://www.ces.ncsu.edu/drought/dro-29.html>).

In many cases, the economic effects relative to reduced productivity of the animals, increased occurrence of disease by immune suppression, damage to vital organs and an adverse effect on reproductivity are <sup>even</sup> greater than the effects caused by death of the animals by mycotoxin intoxication.

<sup>A</sup> The group of aflatoxins <sup>can be adsorbed</sup> is ~~fixed~~ <sup>by</sup> with high specificity ~~on~~ <sup>others</sup> some ~~minimal~~ absorbents, like zeolite, bentonite, aluminum silicate and ~~other~~, because of their specific molecular structure (cf. A. J. Ramos, J. Fink-Gremmels, E. Hernandez, "Prevention of Toxic Effects of Mycotoxins by Means of Non-nutritive Adsorbent Compounds", J. of Food Protection, Vol. 59(6), 1996, page 631-641). However, this is not true for most other mycotoxins. An attempt has been made to expand the adsorption capacity of mineral adsorbents <sup>for</sup> ~~to~~ non-aflatoxins as well.

A dry particulate animal feed additive is described in WO 91/13555, which contains phyllosilicate particles that are coated with a sequestering agent. An increase in sorption rate can be achieved by this <sup>process</sup> ~~but~~ complete (> 90%) elimination of the introduced toxins cannot be achieved. <sup>also</sup> Good results are described in the prior art with ion exchange resins or high-quality activated carbon, but such solutions are not <sup>practical</sup> ~~relevant to practice~~ for cost reasons.

Organophilic clays are used, among other things, in the prior art, to treat liquid wastes with organic contaminants, in order to solidify them and facilitate their disposal (cf. EP-0,560,423).

S. L. Lemke, P. G. Grant and T. D. Phillips describe in "Adsorption of Zearalenone by Organophilic Montmorillonite Clay", J. Agric. Food Chem. (1998), pages 3787-3796 an organically modified acid montmorillonite clay, which is capable of adsorbing zearalenone. The best adsorption rates were exhibited by clays that were exchanged with cations containing C<sub>16</sub> alkyl groups, namely, hexadecyltrimethylammonium (HDTMA) and cetylpyridinium (CP). Noticeable adsorption rates were only achieved from a coating <sup>(with a cation exchange capacity (CEC))</sup> of more than about 75% ~~of the cation exchange capacity (CEC)~~.

The use of organically modified clay for adsorption of fumonisine B1 is described in Lemke, S. L., Ottinger, S. E. and Phillips, T. D., Book of Abstracts, 216<sup>th</sup> ACS National Meeting, Boston,

1998. Quaternary ammonium compounds having a C<sub>16</sub> alkyl group are used for organophilization.

The task of the present invention is to prepare an adsorbent based on layered silicates (phyllosilicates) that adsorbs not only aflatoxins, but also other important mycotoxins (non-aflatoxins) with high efficiency and, at the same time, is ~~so~~ cost-effective <sup>so</sup> that it can be used in practice. The adsorbent ~~is also supposed to exhibit~~ <sup>exhibits</sup> stable adsorption of mycotoxins under physiological conditions, as occur, for example, after absorption with the feeds in the digestive tract of animals.

### Summary of Invention

It was surprisingly found that, by appropriate modification of a layered silicate or part of it, mycotoxin adsorbents can be produced that can effectively adsorb both aflatoxins and non-aflatoxins, like zearalenone, ochratoxin, deoxynivalenone, T2 toxins or fumonisine, <sup>which</sup> ~~and~~ are also cost-effective.

### Detailed Description of Preferred Embodiment

According to a first aspect of the invention, by modification of a layered silicate with a quaternary onium compound with a long-chain C<sub>10</sub> to C<sub>22</sub> alkyl group and at least one aromatic substituent, <sup>even with</sup> ~~and also~~ <sup>with</sup> relatively limited amounts thereof, a significant increase in adsorption performance of such a material for mycotoxins can ~~already~~ be achieved.

The layered silicates listed in Ullmann's Encyclopedia of Industrial Chemistry, Vol. 21, pages 370-375 (1982) fall under the layered silicates employable <sup>as</sup> ~~in~~ the adsorbent according to the invention. In particular, the activatable natural and synthetic clay minerals, like smectites, including montmorillonite, beidellite, nontronite, volkonskoite, stevensite, hectorite, swinefordite, saponite and sauconite, the vermiculites, illites, mixed layer minerals, palygorskite (attapulgite) and sepiolite, can be used. The two last-named minerals are also called hormites.

According to a preferred variant of the invention, the layered silicate is a three-layered silicate, for example, a naturally occurring smectitic clay, especially a bentonite clay. Swellable layered silicates with a relatively high swelling volume are preferred, in particular, like calcium bentonites with a swelling volume of about 10 mL/g or more, or layered silicates converted by

ion exchange to the  $\text{Na}^+$  form with a swelling volume of about 20 mL/g or more. It is assumed that the specific adsorption performance is positively influenced by the high swellability. However, acid-activated bentonite<sup>S</sup> can also be used.

It was found that very good adsorption performance <sup>can be</sup> is achieved for mycotoxins <sup>even</sup> already at an exchange rate ~~being~~ <sup>when the rate is as low as</sup> well below 75% of the cation exchange capacity (CEC) of the layered silicate. Even ~~during~~ exchange of 2 to 30%, preferably 2 to 15%, especially 2 to 10%, of the CEC, the adsorbents according to the invention exhibit significant adsorption performance for mycotoxins.

According to a preferred variant, for example, a bentonite with a cation exchange capacity from 5 to 100 meq/100 g can be uniformly coated with an amount of onium ions corresponding to about 3 to 15 meq/100 g.

Quaternary ammonium compounds and pyridinium compounds can be used, in particular, as quaternary onium compounds. <sup>With the stipulation that</sup> the quaternary onium compounds contain (at least) a long chain  $\text{C}_{10}$  to  $\text{C}_{22}$  alkyl group and at least one aromatic substituent, <sup>one</sup> All onium compounds suitable for organic modification of layered silicates that are known to one skilled in this field can be used. The quaternary onium compounds can also contain an aralkyl substituent (as aromatic substituent).

According to a preferred variant, stearyl (tallow)-benzyltrimethylammonium chloride ( $\text{C}_{16}$ - $\text{C}_{18}$  DMBA) is used as quaternary ammonium compound. Additional preferred onium compounds are:

- Coconut alkyltrimethylbenzylammonium chloride ( $\text{C}_{12}$ - $\text{C}_{16}$  DMBA)
- Dimethylstearylbenzylammonium chloride ( $\text{C}_{12}$ - $\text{C}_{14}$  DMBA)
- Distearyltrimethylbenzylammonium chloride ( $\text{C}_{16}$ - $\text{C}_{18}$  DMBA)
- Quaternized tallow imidazolinium methosulfate.

The quaternary onium compounds can be used either directly or formed in situ during activation of the layered silicate by combined use of secondary and tertiary amines.



It is assumed that the aromatic group(s) and the long chain alkyl group of the quaternary onium compound cooperate to achieve the advantageous adsorption performance. Without restricting the present invention to a theoretical mechanism, it is assumed that the vicinal or isolated carbonyl groups present in nearly all mycotoxins <sup>interact</sup> ~~participate in interaction~~ with the adsorbents according to the invention.

In addition to improved adsorption performance of mycotoxins, it was also found that the adsorbents according to the invention exhibit an efficient and stable adsorption of mycotoxins during a reduction of the pH values, as occurs, for example, during uptake of feeds in the acid gastric medium of a monogastric animal, or ~~also~~ on transition from an acid to neutral or slightly alkaline pH value, as occurs during passage of the food slurry through the digestive tract.

According to a second aspect of the invention, the mycotoxin adsorbent contains a mixture of an organically modified layered silicate and a <sup>not</sup> ~~non~~ organically modified layered silicate, in which the organically modified layered silicate in the mixture is exchanged at least 75% (referred to the total CEC) with a quaternary onium compound.

The layered silicates used according to this variant of the invention correspond to those mentioned above.

It was found that, according to this variant of the invention, even when quaternary onium compounds containing no aromatic substituents are used, good adsorption performance for mycotoxins can be achieved. In principle, all onium compounds suitable for organic modification of layered silicates that are known to one skilled in this field can be used. However, those quaternary ammonium compounds that have (at least) one long chain C<sub>10</sub>-C<sub>22</sub> alkyl group and preferably at least one aromatic substituent, as described above, are preferred.

Generally, the mixture will contain about 0.1 to 50 wt.%, especially about 0.5 to 20 wt.%, of organically modified layered silicate. It was surprisingly found that, even at a fraction of more than about 2 wt.% organically modified layered silicate in the mixture, almost complete (more than 90%) adsorption of mycotoxins (aflatoxins and non-aflatoxins) occurs even at acid pH

values. It is therefore assumed, without restricting the invention to a theoretical mechanism, that the hydrophobic surface of the organically modified layered silicate and the surface of the unmodified layered silicate interact during effective adsorption and slight desorption of mycotoxins. For example, it is assumed that the aflatoxins primarily bind to the unmodified layered silicate in a mixture of organically modified and unmodified layered silicate, so that the surface of the organically modified layered silicate is available for adsorption of the non-aflatoxins that cannot be adsorbed on the unmodified layered silicate. Good adsorption performance with respect to non-aflatoxins is therefore also guaranteed at relatively high aflatoxin concentrations.

Since the organically modified layered silicate represents the <sup>most costly portion</sup> ~~essential cost factor~~ of the mixture, the smallest possible fraction of organically modified layered silicate is chosen in the mixture under practical conditions, but one in which good adsorption performance is observed. The optimal fraction of organically modified or unmodified layered silicate can be determined in individual cases by one skilled in the art by means of a routine experiment.

According to a preferred variant, however, generally about 0.5 to 30 wt.%, especially to 15 wt.%, <sup>most</sup> especially to 10 wt.%, of organically modified layered silicate is used in the mixture.

Adsorption of mycotoxins in an aqueous solution remains stable even during a reduction of the pH value or a transition from acid to neutral or slightly alkaline pH, as occurs under physiological conditions during digestion of feed, i.e., the desorption rate is low.

Another advantage of the adsorbent mixture according to the invention is that, because of the relatively limited fraction of organically modified layered silicate in the mixture, desired hydrophobic substances, like lipophilic vitamins or essential fatty acids, are only bound to a limited degree to the adsorbent and are therefore available for resorption in the digestive tract.

The same advantage is obtained during relatively limited exchange in the case of use of a partially organically modified layered silicate.

According to another aspect of the invention, a feed additive that contains the adsorbent according to the invention is prepared.

It is also possible to produce premixes that contain a fairly high percentage of more than about 50% organically modified layered silicate <sup>which</sup> and are mixed in a second step to produce an adsorbent according to the invention or a feed additive with an unmodified layered silicate.

The mycotoxin adsorbents according to the invention can contain additional components that appear useful for the corresponding application, for example, feed supplements or agents for (enzymatic) detoxification of mycotoxins.

### Examples

The cation exchange capacity was determined as follows.

5 g of clay was screened through a 63  $\mu\text{m}$  sieve and dried at 110°C. Precisely 2 g was then weighed out and mixed with 100 mL of 2 N  $\text{NH}_4\text{Cl}$  solution. The suspension was boiled under reflux for an hour. After standing for about 16 hours, the  $\text{NH}_4^+$  clay was filtered off via a membrane suction filter and washed with deionized water (about 800 mL) to <sup>remove the</sup> ~~freedom from~~ ions. Detection of <sup>effective elimination of</sup> ~~freedom from~~ ions of the wash water was carried out for  $\text{NH}_4^+$  ions with the Nessler reagent sensitive to this (Merck company). The washed out  $\text{NH}_4^+$  clay was taken up by the filter, dried at 110°C for 2 hours, ground, screened (63  $\mu\text{m}$  sieve) and dried again at 110°C. The  $\text{NH}_4^+$  content of the bentonite was then determined according to Kjeldahl. The CEC of the clay is the  $\text{NH}_4^+$  content of the  $\text{NH}_4^+$  clay determined by the Kjeldahl method. The data are given in meq/100 g of clay.

The invention is now explained by means of the following examples.

The different mycotoxins were acquired as crystalline pure substances (SIGMA AG) and taken up in methanol or acetonitrile (50  $\mu\text{g/mL}$ ). To perform the adsorption experiment, dilutions were produced using buffer solutions (dipotassium hydrogen phosphate + citric acid), each of which contained 100  $\mu\text{g}$  of the different toxins per liter.

### Example 1

A natural Ca bentonite was used for the adsorption experiment, having a cation exchange capacity of 90 meq/100 g. Complete exchange of the interlayer cation (100% of the CEC) occurred according to the prior art (S. L. Lemke, P. G. Grant and T. D. Phillips, "Adsorption of Zearalenone by Organophilic Montmorillonite Clay", J. Agric. Food Chem. (1988), page 3790) with the following quaternary ammonium ions:

CP:	Cetylpyridinium chloride
HDTMA:	Hexadecyltrimethylammonium chloride
SBDMA:	Stearylbenzyltrimethylammonium chloride
ODDBMA:	Octadecyldibenzylmethylammonium chloride

The organophilized bentonites were dried and finely ground, so that the residue on a 90  $\mu$ m sieve was less than 10%. They were then added in an amount of 0.02 wt.% to mycotoxin-containing aqueous solutions (100 mL), each of which contained 100  $\mu$ g of the three mycotoxins aflatoxin B1, ochratoxin A and zearalenone in 1 L of aqueous solution (pH 7).

The suspensions so produced were shaken at room temperature for 1 hour upside down, and then centrifuged for 5 minutes at 1500 rpm. The clear supernatant was extracted with 2 mL hexane and the hexane phase investigated by HPLC for the amounts of toxins remaining in the solution.

HPLC determination occurred under the following conditions:

Column:	Spherisorb ODS-2	125 $\times$ 4 mm
Mobile phase:		
Aflatoxin:	600 mL of a 1 mmol NaCl solution/200 mL acetonitrile/ 200 mL methanol	
Ochratoxin:	570 mL acetonitrile/410 mL water/20 mL acetic acid	
Zearalenone:	570 mL acetonitrile/410 mL water/20 mL acetic acid	
Flow rate:	1.5 mL/min	

Detector: Fluorescence  
Wavelength: EX 365 nm / EM 455 nm  
Furnace temperature: 30°C (aflatoxin, ochratoxin);  
40°C (zearalenone).

The percentage adsorption rates were calculated by means of the results. The obtained results are summarized in Table 1.

Table I

Effect of onium ion of different organoclays on adsorption of mycotoxins

	Aflatoxin B1 Adsorption [%]	Zearalenon Adsorption [%]	Ochratoxin Adsorption [%]
100 % CP-Organoton	65,4	43,5	38,7
100 % HDTMA-Organoton	78,2	45,8	46,1
100 % SBDMA-Organoton	88	78,3	82,5
100 % ODDBMA-Organoton	86,5	82,8	85,4

*all #s w/  
periods*

Zearalenon = Zearalenone

Organoton = Organoclay

It is apparent from Table I that the mycotoxin adsorbents according to the invention adsorbed both aflatoxins and non-aflatoxins much better than the CP and HDTMA organoclays according to the prior art.

### Example 2

The bentonites modified with CP, HDTMA or SBDMA, produced as described in Example 1, were mixed with natural unmodified Ca bentonite (cf. Example 1 above) with comparable particle fineness in the following ratio: 96 wt.% Ca bentonite + 4 wt.% organoclay.

The organophilized bentonites were added to mycotoxin-containing aqueous solutions (100 mL) in an amount of 0.5 wt.%, each of the solutions containing 100 µg of the three mycotoxins aflatoxin B1, ochratoxin A and zearalenone in 1 L of aqueous solution (pH 7).

The suspensions so produced were shaken upside down at room temperature for 1 hour, and then centrifuged for 5 minutes at 1500 rpm. The clear supernatant was extracted with 2 mL hexane and the hexane phase investigated, as in Example 1, by HPLC.

The percentage absorption rates were calculated by means of the results. The obtained results are summarized in Table 2.

Table II

Effect of onium ion of different organoclays in clay mixtures on adsorption of mycotoxins

	Aflatoxin B1 Adsorption [%]	Zearalenon Adsorption [%]	Ochratoxin Adsorption [%]
100 % Ca-Bentonit	90,1	18,3	11,8
96 % Ca-Bentonit + 4 % CP-Organoton	90,3	61,3	57,6
+ 4 % HDTMA-Organoton	89,2	62,4	65,7
+ 4 % SBDMA-Organoton	90,6	90,4	93,2

*all numbers  
with periods*

Zearalenon = Zearalenone

Bentonit = Bentonite

Organoton = Organoclay

It is apparent from Table II that the mycotoxin adsorbent according to the invention, which contains a mixture of unmodified bentonite with SBDMA organically modified bentonite almost fully adsorbed both aflatoxins and the non-aflatoxins in contrast to the adsorbents according to the prior art.

### Example 3

A bentonite modified with SBDMA, produced as described in Example 1, was mixed with natural unmodified Ca bentonite with comparable grain fineness in the weight ratios listed in the following Table III.

The mixtures so obtained were added in an amount of 0.5 wt.% to mycotoxin-containing aqueous solutions (100 mL), each of which contained 100 µg of the three mycotoxins aflatoxin B1, ochratoxin A and zearalenone in 1 L of aqueous solution at pH 3 or pH 7.

The suspensions so produced were shaken upside at room temperature for 1 hour, and then centrifuged for 5 minutes at 1500 rpm. The clear supernatant was extracted with 2 mL hexane and the hexane phase investigated, as described in Example 1, by HPLC.

The percentage adsorption rates were calculated by means of the results. The obtained results are summarized in Table III.

Table III

Mycotoxin adsorption on mixtures of unmodified and organically modified bentonite

	Aflatoxin B1 Adsorption [%]		Zearalenon Adsorption [%]		Ochratoxin Adsorption [%]	
	pH 7	pH 3	pH 7	pH 3	pH 7	pH 3
Anteil SBDMA-Organon in Ca-Bentonit						
0%	90,1	96,1	18,3	29,5	11,8	19,2
2%	92,1	95,4	82	89,8	79,4	84,8
3%	90	96,3	88,9	92,3	90,7	88,5
4%	90,6	96	90,4	91,7	93,2	90,2
6%	91,9	95,8	90,8	93,4	95,5	90,5

*all  
numbers  
with  
periods*

Zearalenon = Zearalenone

Left: Percentage of SBDMA organoclay in Ca bentonite

It is apparent from Table III that a very good adsorption of even the non-aflatoxins could be achieved with just 2 wt.% SBDMA organoclay in the mixture.

#### **Example 4**

An organophilized SBDMA bentonite was produced generally as described in Example 1, less SBDMA being used for modification, in order to achieve uniform exchange at a level of 8% of the CEC of bentonite.

An SBDMA bentonite exchanged to 100% of the CEC, produced as described in Example 1, was also mixed with natural unmodified Ca bentonite with comparable particle fineness in a ratio of 96 wt.% Ca bentonite + 4 wt.% SBDMA organoclay.

500 mg of the different adsorbents were metered into each 100 mL of aqueous toxin solution, which corresponds to an amount of 0.5%, referred to the supplied solution.

The suspensions so produced were shaken upside down at room temperature for 1 hour and then centrifuged for 5 minutes at 1500 rpm. The clear supernatant was extracted with 2 mL hexane and the hexane phase investigated, as described in Example 1, by HPLC.

For the desorption experiments, the solid obtained after centrifuging and separation of the liquid phase was resuspended in 100 mL of a fresh buffer solution with the desired pH value, the suspension shaken upside down at room temperature for 1 hour and treated further as described above.



Table IV

Adsorption/desorption behavior and its influencing by the pH value of the medium

	Adsorption / Desorption an SBDMA -Organoton, belegt mit 8% der KAK	Adsorption / Desorption an Gemisch aus Ca-Bentonit + 4 % SBDMA-Organoton
<b>Aflatoxin B1</b>		
Adsorption bei pH 7	>97,5%	>97,5%
Desorption I bei pH 3	< 2,5%	< 2,5%
Desorption II bei pH 7	< 2,5%	< 2,5%
<b>Ochratoxin</b>		
Adsorption bei pH 7	87,5%	93,2%
Desorption I bei pH 3	7,2%	3,2%
Desorption II bei pH 7	5,1%	4,6%

all numbers  
with periods

Headings, Left to Right:

Adsorption/desorption on SBDMA organoclay coated with 8% CEC;

Adsorption/desorption on mixture of Ca bentonite + 4% SBDMA organoclay

bei = at

It is apparent from the table that very good adsorption rates were achieved at pH 7 both with the SBDMA organoclay exchange to 8% of the CEC and the mixture of 96% Ca bentonite and 4% SBDMA organoclay.

Only very limited desorption occurred both during the reduction in pH value of the medium to pH 3 and subsequent rise of the pH value again to 7. Because of this, stable adsorption is demonstrated on the organoclays and organoclay mixtures according to the invention.

For the nonorganically modified bentonite, the adsorption rate was < 20% for ochratoxin and the overall desorption (I + II) was > 40%. When fully exchanged SBDMA organoclay was used, complete adsorption > 97.5% was achieved for aflatoxin and ochratoxin, the desorptions (I, II) were < 2.5%.

## CLAIMS

1. Mycotoxin adsorbent, containing
  - a) an organically modified (organophilic) layered silicate, in which quaternary onium compounds with at least one long chain  $C_{10}$  to  $C_{22}$  alkyl group and at least one aromatic substituent are used for modification,
  - or
  - b) a mixture of a nonorganically modified layered silicate and a layered silicate organically modified to at least 75%, referred to the total cation exchange capacity (CEC).
2. Mycotoxin adsorbent according to Claim 1, characterized by the fact that a quaternary ammonium compound is used as quaternary onium compound, especially with a  $C_{14}$  to  $C_{18}$  alkyl group.
3. Mycotoxin adsorbent according to Claim 1 or 2, characterized by the fact that stearylbenzyltrimethylammonium chloride, coconut alkyldimethylbenzylammonium chloride, dimethylaurylbenzylammonium chloride, distearyltrimethylbenzylammonium chloride or quaternized tallow imidazolinium methosulfate is used as quaternary onium compound.
4. Mycotoxin adsorbent according to one of the preceding claims, characterized by the fact that a smectitic clay mineral is used as layered silicate.
5. Mycotoxin adsorbent according to one of the preceding claims, characterized by the fact that a montmorillonite-containing clay, especially bentonite, is used as layered silicate.
6. Mycotoxin adsorbent according to one of the Claims 1 a), 2 to 5, characterized by the fact that no more than 75% of the exchangeable cations (CEC) of a layered silicate are exchanged with quaternary onium compounds.

7. Mycotoxin adsorbent according to one of the Claims 1 a), 2 to 6, characterized by the fact that 2 to 30%, preferably 2 to 15%, and especially 2 to 10%, of the exchangeable cations of the layered silicate are exchanged with quaternary onium compounds.

8. Mycotoxin adsorbent according to one of the Claims 1 b), 2 to 5, characterized by the fact that the mixture contains 0.1 to 50 wt.%, especially 0.5 to 20 wt.%, and preferably 0.5 to 10 wt.%, of organically modified layered silicate.

9. Mycotoxin adsorbent according to one of the Claims 1 b), 4, 5, 8, characterized by the fact that quaternary onium compounds with at least one long chain C<sub>10</sub> to C<sub>22</sub> alkyl group and at least one aromatic substituent are used for organic modification.

10. Feed additive containing a mycotoxin adsorbent according to one of the preceding claims.

11. Premix for production of a mycotoxin adsorbent or feed additive according to one of the Claims 1 to 9, containing more than 50% organically modified layered silicate.

12. Use of the mycotoxin adsorbent according to one of the Claims 1 to 9 for adsorption of mycotoxins in feeds.

## Abstract

## Summary

Mycotoxin adsorbents are produced containing an organically modified (organophilic) layered silicate, in which quaternary onium compounds <sup>contain</sup> with at least one ~~chain~~-C<sub>10</sub> to C<sub>22</sub> alkyl group and at least one aromatic substituent ~~are used for modification~~, or containing a mixture of ~~an~~ organically modified silicate and a layered silicate organically modified at least to 75%, <sup>not</sup> ~~referring~~ referred to the total cation exchange capacity (CEC) <sup>of the layered silicate</sup>

Title

Mycotoxin Adsorbent

Background of Invention

1. Field of Invention

The present invention concerns a mycotoxin adsorbent, especially for adsorption of aflatoxins and other mycotoxins (non-aflatoxins) in cereals, grains and animal feeds.

2. Prior Art

The term mycotoxin encompasses a group of toxic substances that are formed by different naturally occurring fungi. About 300 to 400 mycotoxins are now known. Cereals and grains are generally considered the natural environment for these fungi. Whereas some types of fungi develop in the still maturing grain, other types primarily attack grain supplies being stored when a certain minimum moisture and ambient temperature conditions are present.

All so-called mycotoxins have a health-hazardous effect primarily on agricultural animals fed with infected grain, but secondarily on humans as well via the food chain. For example, aflatoxins are responsible for the so-called X-disease of turkeys, which destroyed about 100,000 animals in Great Britain in 1960/61, which had been fed with moldy peanut flour.

Some of the most important mycotoxins are:

Aflatoxins B<sub>1</sub>, B<sub>2</sub>, G<sub>1</sub>, G<sub>2</sub>: these are formed by various *Aspergillus* species. Aflatoxin B<sub>1</sub> is carcinogenic even in microgram amounts and causes stomach and liver damage.

Ochratoxin is formed by *Aspergillus ochraceus* and *Penicillium viridicatum* and causes kidney damage.

Zearalenone is formed by *Fusarium graminearum*, which grows on corn, barley and wheat. It is an estrogen-like substance that causes fertility disorders and is suspected to be carcinogenic.

Fumonisin is formed by fungi of the genus *Fusarium* and has been implicated, among other things, in horse deaths.

T2 toxins and T2-like toxins (tricothecenes) are formed by fungi of the genus *Fusarium*.

Moreover, there are a number of additional mycotoxins, like deoxynivalenol, diacetoxyscirpenol, patuline, citrinine, byssochlamic acid, ochratoxin, sterigmatocystine, moniliformine, ergot alkaloids, ergochrome, cytochalasane, penicillanic acid, zearalenone, rubratoxins, tricothecenes (cf. Römpps, *Chemie-Lexikon*, 8<sup>th</sup> Edition, 1985, page 2888), and others, which occur in concentrations that cause health problems in feeds only in isolated circumstances.

Several different toxins that are recognized as causal agents of health problems in humans and animals can be discovered in different feeds by the utilization of sensitive analysis methods. A number of studies have been able to demonstrate that several toxins can occur simultaneously in feeds. This simultaneous occurrence can significantly influence the toxicity of the mycotoxins. In addition to acute damage to agricultural animals that receive mycotoxin-contaminated feed, health impairment in humans has also been discussed in the literature. Such impairment develops after long-term intake of foods, even weakly contaminated with mycotoxins.

In a recent study of suspected feed samples, aflatoxin, deoxynivalenone or fumonisin were found in more than 70% of the investigated samples (cf. *Understanding and Coping with Effects of Mycotoxins in Live Dog Feed and Forage*, North Carolina Cooperative Extension Service, North Carolina State University; <http://www.ces.ncsu.edu/drought/dro-29.html>).

In many cases, the economic effects relative to reduced productivity of the animals, increased occurrence of disease by immune suppression, damage to vital organs and an adverse effect on reproductivity are even greater than the effects caused by death of the animals by mycotoxin intoxication.

A group of aflatoxins can be adsorbed with high specificity by some absorbents, like zeolite, bentonite, aluminum silicate and others, because of their specific molecular structure (cf. A. J. Ramos, J. Fink-Gremmels, E. Hernandez, Prevention of Toxic Effects of Mycotoxins by Means of Non-nutritive Adsorbent Compounds, J. of Food Protection, Vol. 59(6), 1996, page 631-641). However, this is not true for most other mycotoxins. An attempt has been made to expand the adsorption capacity of mineral adsorbents for non-aflatoxins as well.

A dry particulate animal feed additive is described in WO 91/13555, which contains phyllosilicate particles that are coated with a sequestering agent. An increase in sorption rate can be achieved by this process, but complete (> 90%) elimination of the introduced toxins cannot be achieved.

Good results are also described in the prior art with ion exchange resins or high-quality activated carbon, but such solutions are not practical for cost reasons.

Organophilic clays are used, among other things, in the prior art, to treat liquid wastes with organic contaminants, in order to solidify them and facilitate their disposal (cf. EP-0,560,423).

S. L. Lemke, P. G. Grant and T. D. Phillips describe in Adsorption of Zearalenone by Organophilic Montmorillonite Clay, J. Agric. Food Chem. (1998), pages 3787-3796 an organically modified acid montmorillonite clay, which is capable of adsorbing zearalenone. The best adsorption rates were exhibited by clays that were exchanged with cations containing C<sub>16</sub> alkyl groups, namely, hexadecyltrimethylammonium (HDTMA) and cetylpyridinium (CP). Noticeable adsorption rates were only achieved from a coating with a cation exchange capacity (CEC) of more than about 75%.

The use of organically modified clay for adsorption of fumonisine B1 is described in Lemke, S. L., Ottinger, S. E. and Phillips, T. D., Book of Abstracts, 216<sup>th</sup> ACS National Meeting, Boston, 1998. Quaternary ammonium compounds having a C<sub>16</sub> alkyl group are used for

organophilization.

The task of the present invention is to prepare an adsorbent based on layered silicates (phyllosilicates) that adsorbs not only aflatoxins, but also other important mycotoxins (non-aflatoxins) with high efficiency and, at the same time, is cost-effective so that it can be used in practice. The adsorbent also exhibits stable adsorption of mycotoxins under physiological conditions, as occur, for example, after absorption with the feeds in the digestive tract of animals.

#### Summary of Invention

It was surprisingly found that, by appropriate modification of a layered silicate or part of it, mycotoxin adsorbents can be produced that can effectively adsorb both aflatoxins and non-aflatoxins, like zearalenone, ochratoxin, deoxynivalenone, T2 toxins or fumonisine, which are also cost-effective.

#### Detailed Description of Preferred Embodiment

According to a first aspect of the invention, by modification of a layered silicate with a quaternary onium compound with a long-chain  $C_{10}$  to  $C_{22}$  alkyl group and at least one aromatic substituent, a significant increase in adsorption performance of such a material for mycotoxins can be achieved even with use of relatively limited amounts thereof.

The layered silicates listed in Ullmann's Encyclopedia of Industrial Chemistry, Vol. 21, pages 370-375 (1982) fall under the layered silicates employable as the adsorbent according to the invention. In particular, the activatable natural and synthetic clay minerals, like smectites, including montmorillonite, beidellite, nontronite, volkonskoite, stevensite, hectorite, swinefordite, saponite and sauconite, the vermiculites, illites, mixed layer minerals, palygorskite (attapulgite) and sepiolite, can be used. The two last-named minerals are also called hormites.



According to a preferred variant of the invention, the layered silicate is a three-layered silicate, for example, a naturally occurring smectitic clay, especially a bentonite clay. Swellable layered silicates with a relatively high swelling volume are preferred, in particular, like calcium bentonites with a swelling volume of about 10 mL/g or more, or layered silicates converted by ion exchange to the Na<sup>+</sup> form with a swelling volume of about 20 mL/g or more. It is assumed that the specific adsorption performance is positively influenced by high swellability. However, acid-activated bentonites can also be used.

It was found that very good adsorption performance can be achieved for mycotoxins even at an exchange rate well below 75% of the cation exchange capacity (CEC) of the layered silicate. Even when the exchange rate is as low as 2 to 30%, preferably 2 to 15%, especially 2 to 10%, of the CEC, the adsorbents according to the invention exhibit significant adsorption for mycotoxins.

According to a preferred variant, for example, a bentonite with a cation exchange capacity from 5 to 100 meq/100 g can be uniformly coated with onium ions corresponding to about 3 to 15 meq/100 g.

Quaternary ammonium compounds and pyridinium compounds can be used, in particular, as quaternary onium compounds. All onium compounds are suitable for organic modification of layered silicates that are known to one skilled in this field can be used if the quaternary onium compounds contain (at least) a long chain C<sub>10</sub> to C<sub>22</sub> alkyl group and at least one aromatic substituent. The quaternary onium compounds can also contain an aralkyl substituent (as aromatic substituent).

According to a preferred variant, stearyl (tallow)-benzyltrimethylammonium chloride (C<sub>16</sub>-C<sub>18</sub> DMBA) is used as quaternary ammonium compound. Additional preferred onium compounds are:

Coconut alkyldimethylbenzylammonium chloride (C<sub>12</sub>-C<sub>16</sub> DMBA)

Dimethylaurylbenzylammonium chloride ( $C_{12}$ - $C_{14}$  DMBA)

Distearyl methylbenzylammonium chloride ( $C_{16}$ - $C_{18}$  DMBA)

Quaternized tallow imidazolinium methosulfate.

The quaternary onium compounds can be used either directly or formed in situ during activation of the layered silicate by combined use of secondary and tertiary amines.

It is assumed that the aromatic group(s) and the long chain alkyl group of the quaternary onium compound cooperate to achieve the advantageous adsorption performance. Without restricting the present invention to a theoretical mechanism, it is assumed that the vicinal or isolated carbonyl groups present in nearly all mycotoxins interact with the adsorbents according to the invention.

In addition to improved adsorption performance of mycotoxins, it was also found that the adsorbents according to the invention exhibit an efficient and stable adsorption of mycotoxins during a reduction of the pH values, as occurs, for example, during uptake of feeds in the acid gastric medium of a monogastric animal, or on transition from an acid to neutral or slightly alkaline pH value, as occurs during passage of the food slurry through the digestive tract.

According to a second aspect of the invention, the mycotoxin adsorbent contains a mixture of an organically modified layered silicate and a not organically modified layered silicate, in which the organically modified layered silicate in the mixture is exchanged at least 75% (referred to the total CEC) with a quaternary onium compound.

The layered silicates used according to this variant of the invention correspond to those mentioned above.

It was found that, according to this variant of the invention, even when quaternary onium compounds containing no aromatic substituents are used, good adsorption performance for mycotoxins can be achieved. In principle, all onium compounds suitable for organic

modification of layered silicates that are known to one skilled in this field can be used. However, those quaternary ammonium compounds that have (at least) one long chain  $C_{10}$ - $C_{22}$  alkyl group and preferably at least one aromatic substituent, as described above, are preferred.

Generally, the mixture will contain about 0.1 to 50 wt.%, especially about 0.5 to 20 wt.%, of organically modified layered silicate. It was surprisingly found that, even at a fraction of more than about 2 wt.% organically modified layered silicate in the mixture, almost complete (more than 90%) adsorption of mycotoxins (aflatoxins and non-aflatoxins) occurs even at acid pH values. It is therefore assumed, without restricting the invention to a theoretical mechanism, that the hydrophobic surface of the organically modified layered silicate and the surface of the unmodified layered silicate interact during effective adsorption and slight desorption of mycotoxins. For example, it is assumed that the aflatoxins primarily bind to the unmodified layered silicate in a mixture of organically modified and unmodified layered silicate, so that the surface of the organically modified layered silicate is available for adsorption of the non-aflatoxins that cannot be adsorbed on the unmodified layered silicate. Good adsorption performance with respect to non-aflatoxins is therefore also guaranteed at relatively high aflatoxin concentrations.

Since the organically modified layered silicate represents the most costly portion of the mixture, the smallest possible fraction of organically modified layered silicate is chosen in the mixture under practical conditions, but one in which good adsorption performance is observed. The optimal fraction of organically modified or unmodified layered silicate can be determined in individual cases by one skilled in the art by means of a routine experiment.

According to a preferred variant, however, generally about 0.5 to 30 wt.%, especially to 15 wt.%, most especially to 10 wt.%, of organically modified layered silicate is used in the mixture.

Adsorption of mycotoxins in an aqueous solution remains stable even during a reduction of

the pH value or a transition from acid to neutral or slightly alkaline pH, as occurs under physiological conditions during digestion of feed, i.e., the desorption rate is low.

Another advantage of the adsorbent mixture according to the invention is that, because of the relatively limited fraction of organically modified layered silicate in the mixture, desired hydrophobic substances, like lipophilic vitamins or essential fatty acids, are only bound to a limited degree to the adsorbent and are therefore available for resorption in the digestive tract.

The same advantage is obtained during relatively limited exchange in the case of use of a partially organically modified layered silicate.

According to another aspect of the invention, a feed additive that contains the adsorbent according to the invention is prepared.

It is also possible to produce premixes that contain a fairly high percentage of more than about 50% organically modified layered silicate which are mixed in a second step to produce an adsorbent according to the invention or a feed additive with an unmodified layered silicate.

The mycotoxin adsorbents according to the invention can contain additional components that appear useful for the corresponding application, for example, feed supplements or agents for (enzymatic) detoxification of mycotoxins.

### Examples

The cation exchange capacity was determined as follows.

5 g of clay was screened through a 63  $\mu\text{m}$  sieve and dried at 110°C. Precisely 2 g was then weighed out and mixed with 100 mL of 2 N  $\text{NH}_4\text{Cl}$  solution. The suspension was boiled under reflux for an hour. After standing for about 16 hours, the  $\text{NH}_4^+$  clay was filtered off via a membrane suction filter and washed with deionized water (about 800 mL) to remove the

ions. Detection of effective elimination of ions of the wash water was carried out for  $\text{NH}_4^+$  ions with the Nessler reagent sensitive to this (Merck company). The washed out  $\text{NH}_4^+$  clay was taken up by the filter, dried at  $110^\circ\text{C}$  for 2 hours, ground, screened ( $63\ \mu\text{m}$  sieve) and dried again at  $110^\circ\text{C}$ . The  $\text{NH}_4^+$  content of the bentonite was then determined according to Kjeldahl. The CEC of the clay is the  $\text{NH}_4^+$  content of the  $\text{NH}_4^+$  clay determined by the Kjeldahl method. The data are given in meq/100 g of clay.

The invention is now explained by means of the following examples.

The different mycotoxins were acquired as crystalline pure substances (SIGMA AG) and taken up in methanol or acetonitrile ( $50\ \mu\text{g/mL}$ ). To perform the adsorption experiment, dilutions were produced using buffer solutions (dipotassium hydrogen phosphate + citric acid), each of which contained  $100\ \mu\text{g}$  of the different toxins per liter.

### Example 1

A natural Ca bentonite was used for the adsorption experiment, having a cation exchange capacity of 90 meq/100 g. Complete exchange of the interlayer cation (100% of the CEC) occurred according to the prior art (S. L. Lemke, P. G. Grant and T. D. Phillips, "Adsorption of Zearalenone by Organophilic Montmorillonite Clay", J. Agric. Food Chem. (1988), page 3790) with the following quaternary ammonium ions:

CP:	Cetylpyridinium chloride
HDTMA:	Hexadecyltrimethylammonium chloride
SBDMA:	Stearylbenzyltrimethylammonium chloride
ODDBMA:	Octadecyldibenzyltrimethylammonium chloride

The organophilized bentonites were dried and finely ground, so that the residue on a  $90\ \mu\text{m}$  sieve was less than 10%. They were then added in an amount of 0.02 wt.% to mycotoxin-

containing aqueous solutions (100 mL), each of which contained 100  $\mu$ g of the three mycotoxins aflatoxin B1, ochratoxin A and zearalenone in 1 L of aqueous solution (pH 7).

The suspensions so produced were shaken at room temperature for 1 hour upside down, and then centrifuged for 5 minutes at 1500 rpm. The clear supernatant was extracted with 2 mL hexane and the hexane phase investigated by HPLC for the amounts of toxins remaining in the solution.

HPLC determination occurred under the following conditions:

Column:	Spherisorb ODS-2 125 $\times$ 4 mm
Mobile phase:	
Aflatoxin:	600 mL of a 1 mmol NaCl solution/200 mL acetonitrile/ 200 mL methanol
Ochratoxin:	570 mL acetonitrile/410 mL water/20 mL acetic acid
Zearalenone:	570 mL acetonitrile/410 mL water/20 mL acetic acid
Flow rate:	1.5 mL/min
Detector:	Fluorescence
Wavelength:	EX 365 nm / EM 455 nm
Furnace temperature:	30°C (aflatoxin, ochratoxin); 40°C (zearalenone).

The percentage adsorption rates were calculated by means of the results. The obtained results are summarized in Table 1.

Table I

Effect of onium ion of different organoclays on adsorption of mycotoxins

	<b>Aflatoxin B1 Adsorption [%]</b>	<b>Zearalenon Adsorption [%]</b>	<b>Ochratoxin Adsorption [%]</b>
100% CP-Organoton	65.4	43.5	38.7
100% HDTMA-Organoton	78.2	45.8	46.1
100% SBDMA-Organoton	88	78.3	82.5
100% ODDBMA-Organoton	86.5	82.8	85.4

Zearalenon = Zearalenone

Organoton = Organoclay

It is apparent from Table I that the mycotoxin adsorbents according to the invention adsorbed both aflatoxins and non-aflatoxins much better than the CP and HDTMA organoclays according to the prior art.

### **Example 2**

The bentonites modified with CP, HDTMA or SBDMA, produced as described in Example 1, were mixed with natural unmodified Ca bentonite (cf. Example 1 above) with comparable particle fineness in the following ratio: 96 wt.% Ca bentonite + 4 wt.% organoclay.

The organophilized bentonites were added to mycotoxin-containing aqueous solutions (100 mL) in an amount of 0.5 wt.%, each of the solutions containing 100  $\mu$ g of the three mycotoxins aflatoxin B1, ochratoxin A and zearalenone in 1 L of aqueous solution (pH 7).

The suspensions so produced were shaken upside down at room temperature for 1 hour, and then centrifuged for 5 minutes at 1500 rpm. The clear supernatant was extracted with 2 mL hexane and the hexane phase investigated, as in Example 1, by HPLC.

The percentage absorption rates were calculated by means of the results. The obtained results are summarized in Table 2.

Table II

Effect of onium ion of different organoclays in clay mixtures on adsorption of mycotoxins

	<b>Aflatoxin B1 Adsorption [%]</b>	<b>Zearalenon Adsorption [%]</b>	<b>Ochratoxin Adsorption [%]</b>
100% Ca-Bentonit	90.1	18.3	11.8
96% Ca-Bentonit			
+4% CP-Organoton	90.3	61.3	57.6
+4% HDTMA-Organoton	89.2	62.4	65.7
+4% SBDMA-Organoton	90.6	90.4	93.2

Zearalenon = Zearalenone

Bentonit = Bentonite

Organoton = Organoclay

It is apparent from Table II that the mycotoxin adsorbent according to the invention, which contains a mixture of unmodified bentonite with SBDMA organically modified bentonite almost fully adsorbed both aflatoxins and the non-aflatoxins in contrast to the adsorbents according to the prior art.

### **Example 3**

A bentonite modified with SBDMA, produced as described in Example 1, was mixed with natural unmodified Ca bentonite with comparable grain fineness in the weight ratios listed in the following Table III.

The mixtures so obtained were added in an amount of 0.5 wt.% to mycotoxin-containing aqueous solutions (100 mL), each of which contained 100  $\mu$ g of the three mycotoxins aflatoxin B1, ochratoxin A and zearalenone in 1 L of aqueous solution at pH 3 or pH 7.



The suspensions so produced were shaken upside at room temperature for 1 hour, and then centrifuged for 5 minutes at 1500 rpm. The clear supernatant was extracted with 2 mL hexane and the hexane phase investigated, as described in Example 1, by HPLC.

The percentage adsorption rates were calculated by means of the results. The obtained results are summarized in Table III.

Table III

Mycotoxin adsorption on mixtures of unmodified and organically modified bentonite

	<b>Aflatoxin B1</b> <b>Adsorption [%]</b>		<b>Zearalenon</b> <b>Adsorption[%]</b>		<b>Ochratoxin</b> <b>Adsorption [%]</b>	
	<b>pH 7</b>	<b>pH3</b>	<b>pH7</b>	<b>pH3</b>	<b>pH7</b>	<b>pH3</b>
Anteil SBDMA - Organoton in Ca-Bentonit						
0%	90.1	96.1	18.3	29.5	11.8	19.2
2%	92.1	95.4	82	89.8	79.4	84.8
3%	90	96.3	88.9	92.3	90.7	88.5
4%	90.6	96	90.4	91.7	93.2	90.2
6%	91.9	95.8	90.8	93.4	95.5	90.5

Zearalenon = Zearalenone

*Left:* Percentage of SBDMA organoclay in Ca bentonite

It is apparent from Table III that a very good adsorption of even the non-aflatoxins could be achieved with just 2 wt.% SBDMA organoclay in the mixture.

#### Example 4

An organophilized SBDMA bentonite was produced generally as described in Example 1, less

SBDMA being used for modification, in order to achieve uniform exchange at a level of 8% of the CEC of bentonite.

An SBDMA bentonite exchanged to 100% of the CEC, produced as described in Example 1, was also mixed with natural unmodified Ca bentonite with comparable particle fineness in a ratio of 96 wt.% Ca bentonite + 4 wt.% SBDMA organoclay.

500 mg of the different adsorbents were metered into each 100 mL of aqueous toxin solution, which corresponds to an amount of 0.5%, referred to the supplied solution.

The suspensions so produced were shaken upside down at room temperature for 1 hour and then centrifuged for 5 minutes at 1500 rpm. The clear supernatant was extracted with 2 mL hexane and the hexane phase investigated, as described in Example 1, by HPLC.

For the desorption experiments, the solid obtained after centrifuging and separation of the liquid phase was resuspended in 100 mL of a fresh buffer solution with the desired pH value, the suspension shaken upside down at room temperature for 1 hour and treated further as described above.

Table IV

Adsorption/desorption behavior and its influencing by the pH value of the medium

	<b>Adsorption/Desorption an SBDMA-Organoton, belegt mit 8% der KAK</b>	<b>Adsorption/Desorption an Gernisch aus Ca-Bentonit +4% SBDMA-Organoton</b>
Aflatoxin B1		
Adsorption bei pH7	> 97.5%	>97.5%
Desorption I bei pH3	<2.5%	<2.5%
Desorption II bei pH7	<2.5%	<2.5%
Ochratoxin		
Adsorption bei pH7	87.5%	93.2%
Desorption I bei pH3	7.2%	3.2%
Desorption II bei pH7	5.1%	4.6%

*Headings, Left to Right:*

Adsorption/desorption on SBDMA organoclay coated with 8% CEC;

Adsorption/desorption on mixture of Ca bentonite + 4% SBDMA organoclay

bei = at

It is apparent from the table that very good adsorption rates were achieved at pH 7 both with the SBDMA organoclay exchange to 8% of the CEC and the mixture of 96% Ca bentonite and 4% SBDMA organoclay.

Only very limited desorption occurred both during the reduction in pH value of the medium to pH 3 and subsequent rise of the pH value again to 7. Because of this, stable adsorption is demonstrated on the organoclays and organoclay mixtures according to the invention.

For the nonorganically modified bentonite, the adsorption rate was < 20% for ochratoxin and the overall desorption (I + II) was > 40%. When fully exchanged SBDMA organoclay was used, complete adsorption > 97.5% was achieved for aflatoxin and ochratoxin, the desorptions (I, II) were < 2.5%.

## CLAIMS

13. A mycotoxin adsorbent comprising  
an organically modified (organophilic) layered silicate comprising a quaternary onium compound, wherein said quaternary onium compound includes at least a C<sub>10</sub> to C<sub>22</sub> alkyl group and an aromatic substituent.
14. A mycotoxin adsorbent comprising  
a mixture of a layered silicate, which has not been organically modified, and a layered silicate, which has been organically modified to at least about 75 percent of its total cation exchange capacity (CEC).
15. The mycotoxin adsorbent of Claim 14 wherein the organically modified layered silicate comprises a quaternary onium compound including at least a C<sub>10</sub> to C<sub>22</sub> alkyl group and at least one aromatic substituent.
16. The mycotoxin adsorbent of Claim 13 wherein the C<sub>10</sub> to C<sub>22</sub> alkyl group comprises a C<sub>14</sub> to C<sub>18</sub> alkyl group.
17. The mycotoxin adsorbent of Claim 15 wherein the C<sub>10</sub> to C<sub>22</sub> alkyl group comprises a C<sub>14</sub> to C<sub>18</sub> alkyl group.
18. The mycotoxin adsorbent of Claim 13 wherein the quaternary onium compound is selected from a group consisting of stearylbenzyltrimethylammonium chloride, coconut alkyldimethylbenzylammonium chloride, dimethyl-laurylbenzylammonium chloride, distearyl-methylbenzylammonium chloride or quaternized tallow imidazolinium methosulfate is used as quaternary onium compound.
19. The mycotoxin adsorbent of Claim 15 wherein the quaternary onium compound is

selected from a group consisting of stearylbenzyltrimethylammonium chloride, coconut alkyldimethylbenzylammonium chloride, dimethyl-laurylbenzylammonium chloride, distearyl-methylbenzylammonium chloride or quaternized tallow imidazolinium methosulfate is used as quaternary onium compound.

20. The mycotoxin adsorbent of Claim 13 wherein the organically modified layered silicate comprises a smectite clay mineral.

21. The mycotoxin adsorbent of Claim 14 wherein the organically modified layered silicate comprises a smectite clay mineral.

22. The mycotoxin adsorbent of Claim 13 wherein the organically modified layered silicate comprises a montmorillonite-containing clay.

23. The mycotoxin adsorbent of Claim 14 wherein the organically modified layered silicate comprises a montmorillonite-containing clay.

24. The mycotoxin adsorbent of Claim 13 wherein the organically modified layered silicate comprises a bentonite clay.

25. The mycotoxin adsorbent of Claim 14 wherein the organically modified layered silicate comprises a bentonite clay.

26. The mycotoxin adsorbent of Claim 14 wherein no more than 75 percent of exchangeable cations of the layered silicate which has been organically modified are exchanged with a quaternary onium compound.

27. The mycotoxin adsorbent of Claim 14 wherein about 2 to about 30 percent of the exchangeable cations of the layered silicate which has been organically modified are

exchanged with quaternary onium compounds.

28. The mycotoxin adsorbent of Claim 14 wherein about 2 to about 15 percent of the exchangeable cations of the layered silicate which has been organically modified are exchanged with quaternary onium compounds.

29. The mycotoxin adsorbent of Claim 14 wherein about 2 to about 10 percent of the exchangeable cations of the layered silicate which has been organically modified are exchanged with quaternary onium compounds.

30. The mycotoxin adsorbent of Claim 14 wherein the organically modified layered silicate comprises from about 0.1 to about 50 weight percent of the adsorbent.

31. The mycotoxin adsorbent of Claim 14 wherein the organically modified layered silicate comprises from about 0.5 to about 20 weight percent of the adsorbent.

32. The mycotoxin adsorbent of Claim 14 wherein the organically modified layered silicate comprises from about 0.5 to about 10 weight percent of the adsorbent.

33. A mycotoxin adsorbent comprising  
an organically modified (organophilic) layered silicate comprising a quaternary onium compound, wherein said quaternary onium compound includes at least a C<sub>14</sub> to C<sub>18</sub> alkyl group and an aromatic substituent.

34. A mycotoxin adsorbent comprising  
a mixture of a layered silicate which has not been organically modified and a layered silicate which has been organically modified to at least about 75 percent of its total cation exchange capacity (CED) wherein the organically modified layered silicate includes at least a C<sub>14</sub> to C<sub>18</sub> alkyl group and at least one aromatic substituent.

35. A feed additive comprising a mycotoxin adsorbent which comprises an organically modified (organophilic) layered silicate comprising a quaternary onium compound, wherein said quaternary onium compound includes at least a C<sub>10</sub> to C<sub>22</sub> alkyl group and an aromatic substituent.

36. A feed additive comprising a mycotoxin adsorbent, wherein the mycotoxin adsorbent comprises a mixture of a layered silicate which has not been organically modified and a layered silicate which has been organically modified to at least about 75 percent of its total cation exchange capacity (CEC).

37. A premix for production of a feed additive comprising the mycotoxin adsorbent of Claim 13 containing more than 50 percent organically modified layered silicate.

38. A process for the adsorption of mycotoxins in feeds comprising treating the feeds with the mycotoxin adsorbent of Claim 13.

39. A process for the adsorption of mycotoxins in feeds comprising treating feeds with the mycotoxin adsorbent of Claim 14.

## **Abstract**

Mycotoxin adsorbents are produced containing an organically modified (organophilic) layered silicate, in which quaternary onium compounds contain at least one C<sub>10</sub> to C<sub>22</sub> alkyl group and at least one aromatic substituent, or containing a mixture of not organically modified silicate and a layered silicate organically modified at least to 75%, referring to the total cation exchange capacity (CEC) of the layered silicate.

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TRANSLATION FROM GERMAN

PATENT APPLICATION

Mycotoxin Adsorbent

Description

The present invention concerns a mycotoxin adsorbent, especially for adsorption of aflatoxins and other mycotoxins (non-aflatoxins) in feeds.

Background of the Invention

The term mycotoxin encompasses a group of toxic substances that are formed by different naturally occurring fungi. About 300 to 400 mycotoxins are now known. Cereals and grains are generally considered the natural environment for these fungi. Whereas some types of fungi already develop in the spike in the still maturing grain, other types primarily attack grain supplies being stored when a certain minimum moisture and ambient temperature are present.

All so-called mycotoxins have a health-hazardous effect primarily on agricultural animals fed with infected grain, but secondarily on humans as well via the food chain. For example, aflatoxins are responsible for the so-called X-disease of turkeys, which destroyed about 100,000 animals in Great Britain in 1960/61, which had been fed with moldy peanut flour.

Some of the most important mycotoxins are:

Aflatoxins B<sub>1</sub>, B<sub>2</sub>, G<sub>1</sub>, G<sub>2</sub>: these are formed by various *Aspergillus* species. Aflatoxin B<sub>1</sub> is carcinogenic even in microgram amounts and causes stomach and liver damage.

Ochratoxin is formed by *Aspergillus ochraceus* and *Penicillium viridicatum* and causes kidney damage.

Zearalenone is formed by *Fusarium graminearum*, which grows on corn, barley and wheat. It is an estrogen-like substance that causes fertility disorders and is suspected to be carcinogenic.

Fumonisin is formed by fungi of the genus *Fusarium* and has been implicated, among other things, in horse deaths.

T2 toxins and T2-like toxins (tricothecenes) are formed by fungi of the genus *Fusarium*.

Moreover, there are a number of additional mycotoxins, like deoxynivalenol, diacetoxyscirpenol, patuline, citrinine, byssochlamic acid, ochratoxin, sterigmatocystine, moniliformine, ergot alkaloids, ergochrome, cytochalasane, penicillanic acid, zearalenone, rubratoxins, tricothecenes (cf. Römpps, *Chemie-Lexikon*, 8<sup>th</sup> Edition, 1985, page 2888), and others, which occur, however, only in isolated fashion in concentrations that cause health problems in feeds.

Several different toxins that were recognized as causal agents of health problems in humans and animals could be determined in different feeds by the development of more sensitive analysis methods. A number of studies were able to demonstrate that several toxins can occur simultaneously in feeds. This simultaneous occurrence can significantly influence the toxicity of the mycotoxins. In addition to acute damage to agricultural animals that receive mycotoxin-contaminated feed, health impairment in humans is also being discussed in the literature, which develops by long-term intake of foods weakly contaminated with mycotoxins.

In a recent study of suspected feed samples, aflatoxin, deoxynivalenone or fumonisin were found in more than 70% of the investigated samples (cf. "Understanding and Coping with Effects of Mycotoxins in Life Dog Feed and Forage", North Carolina Cooperative Extension Service, North Carolina State University; <http://www.ces.ncsu.edu/drought/dro-29.html>).

In many cases, the economic effects relative to reduced productivity of the animals, increased occurrence of disease by immune suppression, damage to vital organs and an adverse effect on reproductivity are greater than the effects caused by death of the animals by mycotoxin intoxication.

The group of aflatoxins is fixed with high specificity on some minimal absorbents, like zeolite, bentonite, aluminum silicate and other, because of their specific molecular structure (cf. A. J. Ramos, J. Fink-Gremmels, E. Hernandez, "Prevention of Toxic Effects of Mycotoxins by Means of Non-nutritive Adsorbent Compounds", J. of Food Protection, Vol. 59(6), 1996, page 631-641). However, this is not true for most other mycotoxins. An attempt has been made to expand the adsorption capacity of mineral adsorbents to non-aflatoxins as well.

A dry particulate animal feed additive is described in WO 91/13555, which contains phyllosilicate particles that are coated with a sequestering agent. An increase in sorption rate can be achieved by this, but complete (> 90%) elimination of the introduced toxins cannot be achieved. Good results are described in the prior art with ion exchange resins or high-quality activated carbon, but such solutions are not relevant to practice for cost reasons.

Organophilic clays are used, among other things, in the prior art, to treat liquid wastes with organic contaminants, in order to solidify them and facilitate their disposal (cf. EP-0,560,423).

S. L. Lemke, P. G. Grant and T. D. Phillips describe in "Adsorption of Zearalenone by Organophilic Montmorillonite Clay", J. Agric. Food Chem. (1998), pages 3787-3796 an organically modified acid montmorillonite clay, which is capable of adsorbing zearalenone. The best adsorption rates were exhibited by clays that were exchanged with cations containing C<sub>16</sub> alkyl groups, namely, hexadecyltrimethylammonium (HDTMA) and cetylpyridinium (CP). Noticeable adsorption rates were only achieved from a coating of more than about 75% of the cation exchange capacity (CEC).

The use of organically modified clay for adsorption of fumonisine B1 is described in Lemke, S. L., Ottinger, S. E. and Phillips, T. D., Book of Abstracts, 216<sup>th</sup> ACS National Meeting, Boston,

1998. Quaternary ammonium compounds having a C<sub>16</sub> alkyl group are used for organophilization.

The task of the present invention is to prepare an adsorbent based on layered silicates (phyllosilicates) that adsorbs not only aflatoxins, but also other important mycotoxins (non-aflatoxins) with high efficiency and, at the same time, is so cost-effective that it can be used in practice. The adsorbent is also supposed to exhibit stable adsorption of mycotoxins under physiological conditions, as occur, for example, after absorption with the feeds in the digestive tract of animals.

It was surprisingly found that, by appropriate modification of a layered silicate or part of it, mycotoxin adsorbents can be produced that can effectively adsorb both aflatoxins and non-aflatoxins, like zearalenone, ochratoxin, deoxynivalenone, T2 toxins or fumonisine, and are also cost-effective.

According to a first aspect of the invention, by modification of a layered silicate with a quaternary onium compound with a long-chain C<sub>10</sub> to C<sub>22</sub> alkyl group and at least one aromatic substituent, and also with relatively limited amounts thereof, a significant increase in adsorption performance of such a material for mycotoxins can already be achieved.

The layered silicates listed in Ullmann's Encyclopedia of Industrial Chemistry, Vol. 21, pages 370-375 (1982) fall under the layered silicates employable in the adsorbent according to the invention. In particular, the activatable natural and synthetic clay minerals, like smectites, including montmorillonite, beidellite, nontronite, volkonskoite, stevensite, hectorite, swinefordite, saponite and sauconite, the vermiculites, illites, mixed layer minerals, palygorskite (attapulgitite) and sepiolite, can be used. The two last-named minerals are also called hormites.

According to a preferred variant of the invention, the layered silicate is a three-layered silicate, for example, a naturally occurring smectitic clay, especially a bentonite clay. Swellable layered silicates with a relatively high swelling volume are preferred, in particular, like calcium bentonites with a swelling volume of about 10 mL/g or more, or layered silicates converted by

ion exchange to the  $\text{Na}^+$  form with a swelling volume of about 20 mL/g or more. It is assumed that the specific adsorption performance is positively influenced by the high swellability. However, acid-activated bentonite can also be used.

It was found that very good adsorption performance is achieved for mycotoxins already at an exchange rate lying well below 75% of the cation exchange capacity (CEC) of the layered silicate. Even during exchange of 2 to 30%, preferably 2 to 15%, especially 2 to 10%, of the CEC, the adsorbents according to the invention exhibit significant adsorption performance for mycotoxins.

According to a preferred variant, for example, a bentonite with a cation exchange capacity from 5 to 100 meq/100 g can be uniformly coated with an amount of onium ions corresponding to about 3 to 15 meq/100 g.

Quaternary ammonium compounds and pyridinium compounds can be used, in particular, as quaternary onium compounds. With the stipulation that the quaternary onium compounds contain (at least) a long chain  $\text{C}_{10}$  to  $\text{C}_{22}$  alkyl group and at least one aromatic substituent, all onium compounds suitable for organic modification of layered silicates that are known to one skilled in this field can be used. The quaternary onium compounds can also contain an aralkyl substituent (as aromatic substituent).

According to a preferred variant, stearyl (tallow)-benzyltrimethylammonium chloride ( $\text{C}_{16}$ - $\text{C}_{18}$  DMBA) is used as quaternary ammonium compound. Additional preferred onium compounds are:

Coconut alkyldimethylbenzylammonium chloride ( $\text{C}_{12}$ - $\text{C}_{16}$  DMBA)

Dimethylaurylbenzylammonium chloride ( $\text{C}_{12}$ - $\text{C}_{14}$  DMBA)

Distearyltrimethylbenzylammonium chloride ( $\text{C}_{16}$ - $\text{C}_{18}$  DMBA)

Quaternized tallow imidazolinium methosulfate.

The quaternary onium compounds can be used either directly or formed in situ during activation of the layered silicate by combined use of secondary and tertiary amines.

It is assumed that the aromatic group(s) and the long chain alkyl group of the quaternary onium compound cooperate to achieve the advantageous adsorption performance. Without restricting the present invention to a theoretical mechanism, it is assumed that the vicinal or isolated carbonyl groups present in nearly all mycotoxins participate in interaction with the adsorbents according to the invention.

In addition to improved adsorption performance of mycotoxins, it was also found that the adsorbents according to the invention exhibit an efficient and stable adsorption of mycotoxins during a reduction of the pH values, as occurs, for example, during uptake of feeds in the acid gastric medium of a monogastric animal, or also on transition from an acid to neutral or slightly alkaline pH value, as occurs during passage of the food slurry through the digestive tract.

According to a second aspect of the invention, the mycotoxin adsorbent contains a mixture of an organically modified layered silicate and a non-organically modified layered silicate, in which the organically modified layered silicate in the mixture is exchanged at least 75% (referred to the total CEC) with a quaternary onium compound.

The layered silicates used according to this variant of the invention correspond to those mentioned above.

It was found that, according to this variant of the invention, even when quaternary onium compounds containing no aromatic substituents are used, good adsorption performance for mycotoxins can be achieved. In principle, all onium compounds suitable for organic modification of layered silicates that are known to one skilled in this field can be used. However, those quaternary ammonium compounds that have (at least) one long chain C<sub>10</sub>-C<sub>22</sub> alkyl group and preferably at least one aromatic substituent, as described above, are preferred.

Generally, the mixture will contain about 0.1 to 50 wt.%, especially about 0.5 to 20 wt.%, of organically modified layered silicate. It was surprisingly found that, even at a fraction of more than about 2 wt.% organically modified layered silicate in the mixture, almost complete (more than 90%) adsorption of mycotoxins (aflatoxins and non-aflatoxins) occurs even at acid pH

values. It is therefore assumed, without restricting the invention to a theoretical mechanism, that the hydrophobic surface of the organically modified layered silicate and the surface of the unmodified layered silicate interact during effective adsorption and slight desorption of mycotoxins. For example, it is assumed that the aflatoxins primarily bind to the unmodified layered silicate in a mixture of organically modified and unmodified layered silicate, so that the surface of the organically modified layered silicate is available for adsorption of the non-aflatoxins that cannot be adsorbed on the unmodified layered silicate. Good adsorption performance with respect to non-aflatoxins is therefore also guaranteed at relatively high aflatoxin concentrations.

Since the organically modified layered silicate represents the essential cost factor of the mixture, the smallest possible fraction of organically modified layered silicate is chosen in the mixture under practical conditions, but one in which good adsorption performance is observed. The optimal fraction of organically modified or unmodified layered silicate can be determined in individual cases by one skilled in the art by means of a routine experiment.

According to a preferred variant, however, generally about 0.5 to 30 wt.%, especially to 15 wt.%, especially to 10 wt.%, of organically modified layered silicate is used in the mixture.

Adsorption of mycotoxins in an aqueous solution remains stable even during a reduction of the pH value or a transition from acid to neutral or slightly alkaline pH, as occurs under physiological conditions during digestion of feed, i.e., the desorption rate is low.

Another advantage of the adsorbent mixture according to the invention is that, because of the relatively limited fraction of organically modified layered silicate in the mixture, desired hydrophobic substances, like lipophilic vitamins or essential fatty acids, are only bound to a limited degree to the adsorbent and are therefore available for resorption in the digestive tract.

The same advantage is obtained during relatively limited exchange in the case of use of a partially organically modified layered silicate.

According to another aspect of the invention, a feed additive that contains the adsorbent according to the invention is prepared.

It is also possible to produce premixes that contain a fairly high percentage of more than about 50% organically modified layered silicate and are mixed in a second step to produce an adsorbent according to the invention or a feed additive with an unmodified layered silicate.

The mycotoxin adsorbents according to the invention can contain additional components that appear useful for the corresponding application, for example, feed supplements or agents for (enzymatic) detoxification of mycotoxins.

The cation exchange capacity was determined as follows.

5 g of clay was screened through a 63  $\mu\text{m}$  sieve and dried at 110°C. Precisely 2 g was then weighed out and mixed with 100 mL of 2 N  $\text{NH}_4\text{Cl}$  solution. The suspension was boiled under reflux for an hour. After standing for about 16 hours, the  $\text{NH}_4^+$  clay was filtered off via a membrane suction filter and washed with deionized water (about 800 mL) to freedom from ions. Detection of freedom from ions of the wash water was carried out for  $\text{NH}_4^+$  ions with the Nessler reagent sensitive to this (Merck company). The washed out  $\text{NH}_4^+$  clay was taken up by the filter, dried at 110°C for 2 hours, ground, screened (63  $\mu\text{m}$  sieve) and dried again at 110°C. The  $\text{NH}_4^+$  content of the bentonite was then determined according to Kjeldahl. The CEC of the clay is the  $\text{NH}_4^+$  content of the  $\text{NH}_4^+$  clay determined by the Kjeldahl method. The data are given in meq/100 g of clay.

The invention is now explained by means of the following examples.

The different mycotoxins were acquired as crystalline pure substances (SIGMA AG) and taken up in methanol or acetonitrile (50  $\mu\text{g/mL}$ ). To perform the adsorption experiment, dilutions were produced using buffer solutions (dipotassium hydrogen phosphate + citric acid), each of which contained 100  $\mu\text{g}$  of the different toxins per liter.



### Example 1

A natural Ca bentonite was used for the adsorption experiment, having a cation exchange capacity of 90 meq/100 g. Complete exchange of the interlayer cation (100% of the CEC) occurred according to the prior art (S. L. Lemke, P. G. Grant and T. D. Phillips, "Adsorption of Zearalenone by Organophilic Montmorillonite Clay", J. Agric. Food Chem. (1988), page 3790) with the following quaternary ammonium ions:

CP:	Cetylpyridinium chloride
HDTMA:	Hexadecyltrimethylammonium chloride
SBDMA:	Stearylbenzyltrimethylammonium chloride
ODDBMA:	Octadecyldibenzyltrimethylammonium chloride

The organophilized bentonites were dried and finely ground, so that the residue on a 90  $\mu$ m sieve was less than 10%. They were then added in an amount of 0.02 wt.% to mycotoxin-containing aqueous solutions (100 mL), each of which contained 100  $\mu$ g of the three mycotoxins aflatoxin B<sub>1</sub>, ochratoxin A and zearalenone in 1 L of aqueous solution (pH 7).

The suspensions so produced were shaken at room temperature for 1 hour upside down, and then centrifuged for 5 minutes at 1500 rpm. The clear supernatant was extracted with 2 mL hexane and the hexane phase investigated by HPLC for the amounts of toxins remaining in the solution.

HPLC determination occurred under the following conditions:

Column:	Spherisorb ODS-2	125 $\times$ 4 mm
Mobile phase:		
Aflatoxin:	600 mL of a 1 mmol NaCl solution/200 mL acetonitrile/ 200 mL methanol	
Ochratoxin:	570 mL acetonitrile/410 mL water/20 mL acetic acid	
Zearalenone:	570 mL acetonitrile/410 mL water/20 mL acetic acid	
Flow rate:	1.5 mL/min	

Detector: Fluorescence  
Wavelength: EX 365 nm / EM 455 nm  
Furnace temperature: 30°C (aflatoxin, ochratoxin);  
40°C (zearalenone).

The percentage adsorption rates were calculated by means of the results. The obtained results are summarized in Table 1.

Table I

Effect of onium ion of different organoclays on adsorption of mycotoxins

	Aflatoxin B1 Adsorption [%]	Zearalenon Adsorption [%]	Ochratoxin Adsorption [%]
100 % CP-Organoton	65,4	43,5	38,7
100 % HDTMA-Organoton	78,2	45,8	46,1
100 % SBDMA-Organoton	88	78,3	82,5
100 % ODDBMA-Organoton	86,5	82,8	85,4

Zearalenon = Zearalenone

Organoton = Organoclay

It is apparent from Table I that the mycotoxin adsorbents according to the invention adsorbed both aflatoxins and non-aflatoxins much better than the CP and HDTMA organoclays according to the prior art.

### Example 2

The bentonites modified with CP, HDTMA or SBDMA, produced as described in Example 1, were mixed with natural unmodified Ca bentonite (cf. Example 1 above) with comparable particle fineness in the following ratio: 96 wt.% Ca bentonite + 4 wt.% organoclay.

The organophilized bentonites were added to mycotoxin-containing aqueous solutions (100 mL) in an amount of 0.5 wt.%, each of the solutions containing 100 µg of the three mycotoxins aflatoxin B1, ochratoxin A and zearalenone in 1 L of aqueous solution (pH 7).

The suspensions so produced were shaken upside down at room temperature for 1 hour, and then centrifuged for 5 minutes at 1500 rpm. The clear supernatant was extracted with 2 mL hexane and the hexane phase investigated, as in Example 1, by HPLC.

The percentage absorption rates were calculated by means of the results. The obtained results are summarized in Table 2.

Table II

Effect of onium ion of different organoclays in clay mixtures on adsorption of mycotoxins

	Aflatoxin B1 Adsorption [%]	Zearalenon Adsorption [%]	Ochratoxin Adsorption [%]
100 % Ca-Bentonit	90,1	18,3	11,8
96 % Ca-Bentonit + 4 % CP-Organoton	90,3	61,3	57,6
+ 4 % HDTMA-Organoton	89,2	62,4	65,7
+ 4 % SBDMA-Organoton	90,6	90,4	93,2

Zearalenon = Zearalenone

Bentonit = Bentonite

Organoton = Organoclay

It is apparent from Table II that the mycotoxin adsorbent according to the invention, which contains a mixture of unmodified bentonite with SBDMA organically modified bentonite almost fully adsorbed both aflatoxins and the non-aflatoxins in contrast to the adsorbents according to the prior art.

### Example 3

A bentonite modified with SBDMA, produced as described in Example 1, was mixed with natural unmodified Ca bentonite with comparable grain fineness in the weight ratios listed in the following Table III.

The mixtures so obtained were added in an amount of 0.5 wt.% to mycotoxin-containing aqueous solutions (100 mL), each of which contained 100 µg of the three mycotoxins aflatoxin B1, ochratoxin A and zearalenone in 1 L of aqueous solution at pH 3 or pH 7.

The suspensions so produced were shaken upside at room temperature for 1 hour, and then centrifuged for 5 minutes at 1500 rpm. The clear supernatant was extracted with 2 mL hexane and the hexane phase investigated, as described in Example 1, by HPLC.

The percentage adsorption rates were calculated by means of the results. The obtained results are summarized in Table III.

Table III

Mycotoxin adsorption on mixtures of unmodified and organically modified bentonite

	Aflatoxin B1 Adsorption [%]		Zearalenon Adsorption [%]		Ochratoxin Adsorption [%]	
	pH 7	pH 3	pH 7	pH 3	pH 7	pH 3
Anteil SBDMA-Organon in Ca-Bentonit						
0%	90,1	96,1	18,3	29,5	11,8	19,2
2%	92,1	95,4	82	89,8	79,4	84,8
3%	90	96,3	88,9	92,3	90,7	88,5
4%	90,6	96	90,4	91,7	93,2	90,2
6%	91,9	95,8	90,8	93,4	95,5	90,5

Zearalenon = Zearalenone

Left: Percentage of SBDMA organoclay in Ca bentonite

It is apparent from Table III that a very good adsorption of even the non-aflatoxins could be achieved with just 2 wt.% SBDMA organoclay in the mixture.

#### **Example 4**

An organophilized SBDMA bentonite was produced generally as described in Example 1, less SBDMA being used for modification, in order to achieve uniform exchange at a level of 8% of the CEC of bentonite.

An SBDMA bentonite exchanged to 100% of the CEC, produced as described in Example 1, was also mixed with natural unmodified Ca bentonite with comparable particle fineness in a ratio of 96 wt.% Ca bentonite + 4 wt.% SBDMA organoclay.

500 mg of the different adsorbents were metered into each 100 mL of aqueous toxin solution, which corresponds to an amount of 0.5%, referred to the supplied solution.

The suspensions so produced were shaken upside down at room temperature for 1 hour and then centrifuged for 5 minutes at 1500 rpm. The clear supernatant was extracted with 2 mL hexane and the hexane phase investigated, as described in Example 1, by HPLC.

For the desorption experiments, the solid obtained after centrifuging and separation of the liquid phase was resuspended in 100 mL of a fresh buffer solution with the desired pH value, the suspension shaken upside down at room temperature for 1 hour and treated further as described above.

Table IV

Adsorption/desorption behavior and its influencing by the pH value of the medium

	Adsorption / Desorption an SBDMA -Organoton, belegt mit 8% der KAK	Adsorption / Desorption an Gemisch aus Ca-Bentonit + 4 % SBDMA-Organoton
<b>Aflatoxin B1</b>		
Adsorption bei pH 7	>97,5%	>97,5%
Desorption I bei pH 3	< 2,5%	< 2,5%
Desorption II bei pH 7	< 2,5%	< 2,5%
<b>Ochratoxin</b>		
Adsorption bei pH 7	87,5%	93,2%
Desorption I bei pH 3	7,2%	3,2%
Desorption II bei pH 7	5,1%	4,6%

*Headings, Left to Right:*

Adsorption/desorption on SBDMA organoclay coated with 8% CEC;

Adsorption/desorption on mixture of Ca bentonite + 4% SBDMA organoclay

bei = at

It is apparent from the table that very good adsorption rates were achieved at pH 7 both with the SBDMA organoclay exchange to 8% of the CEC and the mixture of 96% Ca bentonite and 4% SBDMA organoclay.

Only very limited desorption occurred both during the reduction in pH value of the medium to pH 3 and subsequent rise of the pH value again to 7. Because of this, stable adsorption is demonstrated on the organoclays and organoclay mixtures according to the invention.

For the nonorganically modified bentonite, the adsorption rate was < 20% for ochratoxin and the overall desorption (I + II) was > 40%. When fully exchanged SBDMA organoclay was used, complete adsorption > 97.5% was achieved for aflatoxin and ochratoxin, the desorptions (I, II) were < 2.5%.

## CLAIMS

1. Mycotoxin adsorbent, containing
  - a) an organically modified (organophilic) layered silicate, in which quaternary onium compounds with at least one long chain C<sub>10</sub> to C<sub>22</sub> alkyl group and at least one aromatic substituent are used for modification,
  - or
  - b) a mixture of a nonorganically modified layered silicate and a layered silicate organically modified to at least 75%, referred to the total cation exchange capacity (CEC).
2. Mycotoxin adsorbent according to Claim 1, characterized by the fact that a quaternary ammonium compound is used as quaternary onium compound, especially with a C<sub>14</sub> to C<sub>18</sub> alkyl group.
3. Mycotoxin adsorbent according to Claim 1 or 2, characterized by the fact that stearylbenzyltrimethylammonium chloride, coconut alkyltrimethylbenzylammonium chloride, dimethylaurylbenzylammonium chloride, distearyltrimethylbenzylammonium chloride or quaternized tallow imidazolinium methosulfate is used as quaternary onium compound.
4. Mycotoxin adsorbent according to one of the preceding claims, characterized by the fact that a smectitic clay mineral is used as layered silicate.
5. Mycotoxin adsorbent according to one of the preceding claims, characterized by the fact that a montmorillonite-containing clay, especially bentonite, is used as layered silicate.
6. Mycotoxin adsorbent according to one of the Claims 1 a), 2 to 5, characterized by the fact that no more than 75% of the exchangeable cations (CEC) of a layered silicate are exchanged with quaternary onium compounds.

7. Mycotoxin adsorbent according to one of the Claims 1 a), 2 to 6, characterized by the fact that 2 to 30%, preferably 2 to 15%, and especially 2 to 10%, of the exchangeable cations of the layered silicate are exchanged with quaternary onium compounds.

8. Mycotoxin adsorbent according to one of the Claims 1 b), 2 to 5, characterized by the fact that the mixture contains 0.1 to 50 wt.%, especially 0.5 to 20 wt.%, and preferably 0.5 to 10 wt.%, of organically modified layered silicate.

9. Mycotoxin adsorbent according to one of the Claims 1 b), 4, 5, 8, characterized by the fact that quaternary onium compounds with at least one long chain  $C_{10}$  to  $C_{22}$  alkyl group and at least one aromatic substituent are used for organic modification.

10. Feed additive containing a mycotoxin adsorbent according to one of the preceding claims.

11. Premix for production of a mycotoxin adsorbent or feed additive according to one of the Claims 1 to 9, containing more than 50% organically modified layered silicate.

12. Use of the mycotoxin adsorbent according to one of the Claims 1 to 9 for adsorption of mycotoxins in feeds.



### Summary

Mycotoxin adsorbents are produced containing an organically modified (organophilic) layered silicate, in which quaternary onium compounds with at least one chain  $C_{10}$  to  $C_{22}$  alkyl group and at least one aromatic substituent are used for modification, or containing a mixture of nonorganically modified silicate and a layered silicate organically modified at least to 75%, referred to the total cation exchange capacity (CEC).

**COMBINED DECLARATION FOR PATENT APPLICATION AND POWER OF ATTORNEY**

(Includes Reference to PCT International Applications)

ATTORNEY'S DOCKET NUMBER

P-1067

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name.

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

MYKOTOXIN ADSORBENS

the specification of which (check only one item below):

☐ is attached hereto.☐ was filed as United States application

Serial No. \_\_\_\_\_

on \_\_\_\_\_

and was amended

on \_\_\_\_\_ (if applicable).

☒ was filed as PCT international application

Number PCT/EP 99/10088

on 17 December 1999

and was amended under PCT Article 19

on \_\_\_\_\_ (if applicable).

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to the examination of this application in accordance with Title 37, Code of Federal Regulations, §1.56(a).

I hereby claim foreign priority benefits under Title 35, United States Code, §119 of any foreign application(s) for patent or inventor's certificate or of any PCT international application(s) designating at least one country other than the United States of America listed below and have also identified below any foreign application(s) for patent or inventor's certificate or any PCT international application(s) designating at least one country other than the United States of America filed by me on the same subject matter having a filing date before that of the application(s) of which priority is claimed:

**PRIOR FOREIGN/PCT APPLICATION(S) AND ANY PRIORITY CLAIMS UNDER 35 U.S.C. 119:**

COUNTRY (If PCT indicate PCT)	APPLICATION NUMBER	DATE OF FILING (day month, year)	PRIORITY CLAIMED UNDER 35 USC 119
PCT	PCT/EP 99/10088	17 Dec. 1999	<input checked="" type="checkbox"/> YES <input type="checkbox"/> NO
Germany	DE 199008132	12 Jan. 1999	<input checked="" type="checkbox"/> YES <input type="checkbox"/> NO
			<input type="checkbox"/> YES <input type="checkbox"/> NO
			<input type="checkbox"/> YES <input type="checkbox"/> NO
			<input type="checkbox"/> YES <input type="checkbox"/> NO

Combined Declaration For Patent Application and Power of Attorney (Continued)				ATTORNEY'S DOCKET NUMBER P-1067
<p>I hereby claim the benefit under Title 35, United States Code, §120 of any United States application(s) or PCT international application(s) designating the United States of America that is/are listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in that/those prior application(s) in the manner provided by the first paragraph of Title 35, United States Code, §112, I acknowledge the duty to disclose material information as defined in Title 37, Code of Federal Regulations, §1.56(a) which occurred between the filing date of the prior application(s) and the national or PCT international filing date of this application.</p>				
<b>PRIOR U.S. APPLICATIONS OR PCT INTERNATIONAL APPLICATIONS DESIGNATING THE U.S. FOR BENEFIT UNDER 35 U.S.C. 120</b>				
U.S. APPLICATIONS			STATUS (Check one)	
U.S. APPLICATION NUMBER	U.S. FILING DATE	PATENTED	PENDING	ABANDONED
PCT APPLICATIONS DESIGNATING THE U.S.				
PCT APPLICATION NO	PCT FILING DATE	U.S. SERIAL NUMBERS ASSIGNED (if any)		
PCT/EP 99/10088	17 Dec. 1999			
<p><b>POWER OF ATTORNEY.</b> As a named inventor, I hereby appoint the following attorney(s) and/or agent(s) to prosecute this application and transact all business in the Patent and Trademark Office connected therewith. (List name and registration number)</p>				
Send Correspondence to <u>Scott R. Cox</u> <u>LYNCH, COX, GILMAN &amp; MAHAN, P.S.C.</u> <u>400 West Market St., Suite 2200</u> <u>Louisville, KY 40202</u>			Direct Telephone Calls to (name and telephone number)  Scott R. Cox 502 589-4215	
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202	FULL NAME OF INVENTOR <u>Simmler-Hübenthal</u> RESIDENCE & CITIZENSHIP CITY <u>Moosburg DEY</u> POST OFFICE ADDRESS <u>Am Mühlbachbogen 13</u>	FAMILY NAME <u>Simmler-Hübenthal</u> CITY <u>Moosburg DEY</u> POST OFFICE ADDRESS <u>Am Mühlbachbogen 13</u>	FIRST GIVEN NAME <u>Hubert</u> STATE OR FOREIGN COUNTRY <u>Germany</u> CITY <u>D-85368 Moosburg</u>	SECOND GIVEN NAME  COUNTRY OF CITIZENSHIP <u>Germany</u> STATE & ZIP CODE/COUNTRY <u>Germany</u>
203	FULL NAME OF INVENTOR <u>Feldhaus</u> RESIDENCE & CITIZENSHIP CITY <u>Pedregal de San Angel</u> POST OFFICE ADDRESS <u>Canada Nr. 250</u>	FAMILY NAME <u>Feldhaus</u> CITY <u>Pedregal de San Angel</u> POST OFFICE ADDRESS <u>Canada Nr. 250</u>	FIRST GIVEN NAME <u>Herrmann</u> STATE OR FOREIGN COUNTRY <u>Mexico MXZ</u> CITY <u>Pedregal de San Angel</u>	SECOND GIVEN NAME <u>Gerardo</u> COUNTRY OF CITIZENSHIP <u>Mexico</u> STATE & ZIP CODE/COUNTRY <u>Mexico D.F. 01900</u>
<p>I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true, and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon</p>				
SIGNATURE OF INVENTOR 201	SIGNATURE OF INVENTOR 202	SIGNATURE OF INVENTOR 203		
DATE <u>12.7.01</u>	DATE <u>17.07.2001</u>	DATE <u>25.07.2001</u>		